



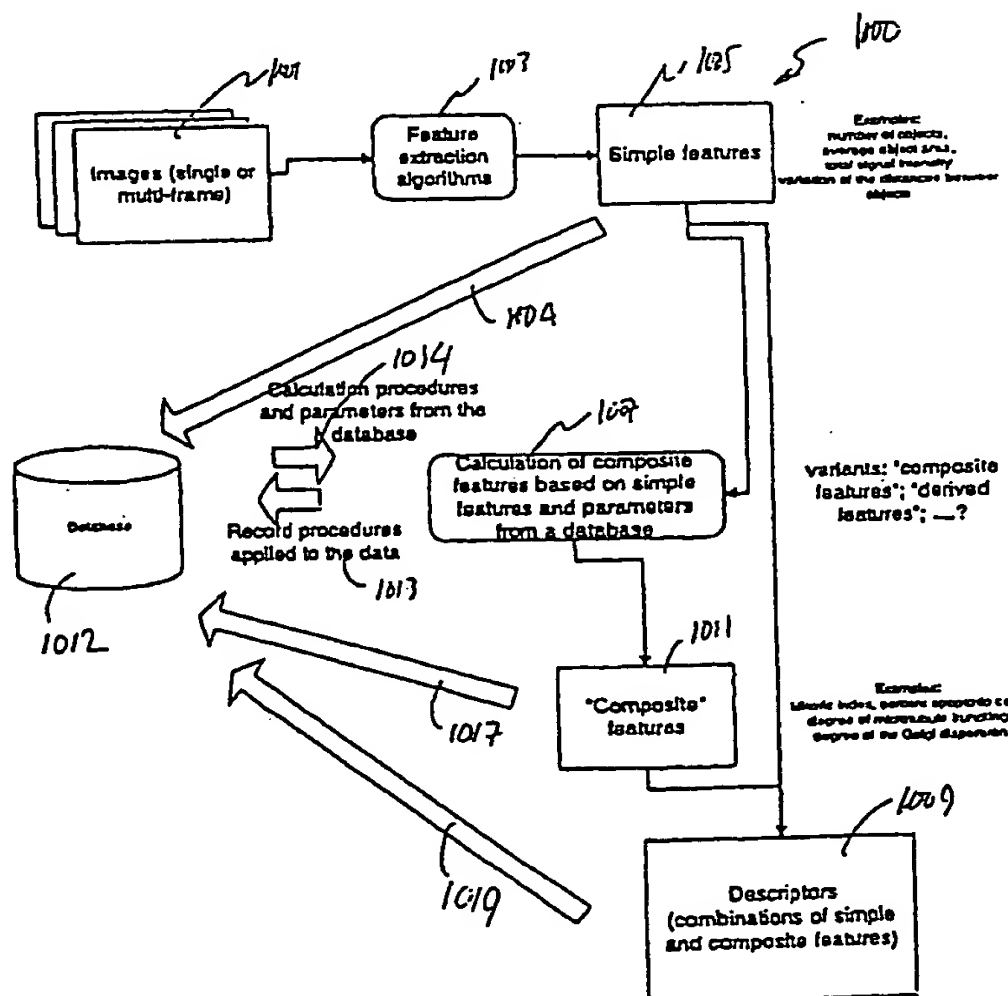
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(57) Abstract

Techniques for using information technology in therapeutics or drug discovery. In an exemplary embodiment, techniques for determining information about the properties of substances based upon information about structure of living or non-living cells exposed to substances are provided. A method according to the present invention enables researchers and/or scientists to identify promising candidates in the search for new and better medicines or treatments using, for example, a cellular informatics database. The present invention further teaches a system for acquiring knowledge from cellular information. The system has a database 1012 comprising a database management module ("DBMS"). The system also has a variety of modules, including a population module coupled to the DBMS for categorizing and storing a plurality of features (e.g., cell size, distance between cells, cell population, cell type) from an image acquisition device into the database. The system has a translation module coupled to the DBMS for defining a descriptor from a set of selected features from the plurality of features. In a specific embodiment, the descriptor is for a known or unknown compound, e.g., drug. A prediction module is coupled to the DBMS for selecting one of a plurality of descriptors from known and unknown compounds from the database based upon a selected descriptor from a selected compound. The selected compound may be one that is useful for treatment of human beings or the like.



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PATENT APPLICATION
METHOD AND APPARATUS FOR
PREDICTIVE CELLULAR BIOINFORMATICS
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10 computer codes, which may be used to implement aspects of the present invention. Assignee of the present invention reserves all rights with respect to these codes and provides notice herein. Notice is hereby given © Cytokinetics, Inc. 1999.

BACKGROUND OF THE INVENTION

 The present invention provides techniques for information
15 management using a database platform. More particularly, the present invention provides a system including computer code that couples to a database device. The system provides for image capturing of living, dead, or fixed cells or cell fractions used to identify information about substances used on the cells or information about the cells themselves. Accordingly, the present invention can enable researchers and
20 scientists to identify promising candidates in the search for new and better medicines, for example, in drug discovery and development. The principles enumerated herein may, with equal facility, be applied to other applications, including but not limited to use in environmental applications such as determining chemical toxicities and other non-pharmaceutical toxicology uses.

25 For a long time, researchers in the pharmaceutical field have sought for better ways of searching for substances possessing properties that make them suitable as medicines. In the early days, researchers generally relied upon extracts from plants, dyes, and microbiological extracts for such substances. Examples of such substances include the pain reliever aspirin, the anti-cancer drug paclitaxel (brand
30 name TaxolTM), and the heart medication called digoxin. The number of useful medicines has generally been limited.

Purified substances having desirable bio-active properties are also often difficult to discover. Advances in traditional organic chemistry and more recently the rapid chemical synthesis methods often referred to as combinatorial chemistry have increased the number of compounds that researchers test for biological activity. Originally, substances were often initially tested on animals or humans to determine their biological activity. While results from such tests may identify a good drug candidate, they are often time consuming and costly, thus a limited number of substances can be tested. Therefore, pharmaceutical companies have turned to testing their ever-increasing libraries of substances against isolated proteins (drug targets) in biochemical assays that can be carried out at high throughput and low cost. It should be noted that the substances need to be tested in numerous protein tests, each customized for a particular drug target. Therefore, although each protein test may be run at a high-throughput, the design of multiple protein tests can be time-consuming. Substances deemed promising based on results from the protein tests are then tested in lower throughput cellular and animal tests.

There have been some attempts to use image acquisition techniques to screen a large number of substances based upon biological cell information. One such attempt is described in International Application No. WO 98/38490 in the names of Dunlay, et al. Dunlay et al. generally describes a conventional image acquisition system. This conventional system collects and saves images based on certain criteria that are predefined, not on a fixed area of an imaging surface. Additionally, the conventional system has poor lighting design, which makes image processing for multiple cells difficult. Furthermore, the conventional system is not designed for capturing, populating and utilizing a large database design. The conventional system is designed for customized cellular assays, not as a tool for generation of a cellular informatics database. Without such database capabilities the conventional system cannot be used for screening, analyzing, and comparing large quantities of cells from multiple experiments on multiple days in a predictive, efficient and cost effective manner.

What is needed is a rapid assay to assess the activity of compounds against multiple drug targets simultaneously in a cellular context. What is also needed are techniques for finding the effects of substances on cell function based upon searching and analyzing cellular information.

SUMMARY OF THE INVENTION

According to at least one embodiment of the present invention, techniques for determining information about effects of potential substances on cells are provided. In another exemplary embodiment, the present invention provides a novel system including hardware, computer codes, user interfaces, and a database for acquiring, storing and retrieving cellular and substance information. The cells can include living, dead, or fixed cells or fractions of cells. The present invention enables, *inter alia*, researchers and/or scientists to identify promising candidates in the search for new and better medicines or treatments using, for example, a cellular informatics database.

According to the present invention, a computer program for identification and verification of biological properties of substances can include code that causes a sample of a substance to be administered to a cell. The code determines one or more features for two or more cell components, or markers, in the presence of the substance. The code can form one or more descriptors from the features. Descriptors can be formed by combining features of two or more cell components as identified using the markers. The code can then search one or more descriptors obtained from prior administered substances upon cells in order to locate descriptors having a relationship to the descriptors noted for the substance under study. The code predicts properties of the administered substance based upon the properties of the prior administered substances using the relationship between the descriptors. The code can provide for identifying properties of substances based upon effects on cell characteristics. Candidate drug mechanisms of action, potency, specificity, pharmacodynamic, and pharmacokinetic parameters, toxicity, and the like can be used as substance properties.

In a specific embodiment, the present invention provides a system for acquiring knowledge from cellular information. The system has a database comprising a database management module ("DBMS"). The system also has a variety of other modules, including a population module that is coupled to the DBMS and serves to categorize and store a plurality of features (including but not limited to cell size, distance between cells, cell population, as well as sub-cellular features such as organelle location, protein location and sub-cellular constituent location and

movement) from an image acquisition device into the database. The system has a translation module coupled to the DBMS for defining a descriptor from a set of selected features from the plurality of features. In a specific embodiment, the descriptor is for a known or unknown compound, e.g., drug. A prediction module is coupled to the DBMS for selecting one of a plurality of a descriptors from known and unknown compounds from the database based upon a selected descriptor from a selected compound. The selected compound may be one that is useful for treatment of human beings or the like.

In a specific embodiment, the present invention provides a system for populating a database with cellular information. The system includes a cell holder (e.g., multi-well plate, chip, microfluidic assembly, or other cell chamber) comprising a plurality of sites in a spatial orientation. Each of the sites is capable of holding a plurality of cells to be imaged. Note – the light guide is one embodiment, but we don't want to be limited to it.

According to one embodiment, the present system also has an illumination apparatus including a liquid light guide operably coupled to the imaging device for highlighting the plurality of cells in a relatively even spatial manner for image capturing and measurement purposes. Still further, the liquid light guide allows sub-elements (e.g., filter, lamp) of the illumination apparatus to be placed at a remote location to prevent mechanical interference of the cell holder during image capturing. Alternative lighting methodologies may, with equal facility, be implemented.

The system also has an image-capturing device (e.g., charge coupled device camera, translation stage, shutter, microscope, software, shutter control) coupled to a computing device (e.g., computer, network computer, work station, analog computing device, on-board image-processor, and laptop). The image-capturing device is adapted to capture at least one image in at least one of the plurality of sites. One some embodiments, multiple images can be captured, where each image represents a different cell component (or portion). The image-capturing device can be adapted to convert the image into a digital representation, which highlights the feature or features of the one site.

A database storage device (e.g., relational database, object oriented database, mixed object oriented database) includes a database management element. The

database is coupled to the image capturing device. In a specific embodiment, the present system includes modules for feature extraction, generation of descriptions, and data preparation and analysis.

In a specific embodiment, the present invention provides a novel
5 system for determining an effect of a manipulation of a cell using one or more image frames. The system has a plate comprising a plurality of sites in a spatial orientation. Each of the sites is capable of holding a plurality of cells to be imaged. The system also has an image capturing device to capture a plurality of images of at least one site from the plurality of sites. The image capturing device is coupled to the computing
10 device. The system also has an image processing device to combine the plurality of images of at least one site or plurality of sites. The image processing device is operably coupled to the plate. An image processing device is also included. The image processing device can be adapted to form a digitized representation of the plurality of images from the site or plurality of sites. Furthermore, the system has a
15 database storage device comprising a database management element. The database can be adapted to retrieve the descriptor or descriptors of the plurality of features from the computing processing device and storing them in a selected manner.

In a specific embodiment, the present invention provides a system for capturing cellular information. The system also has an image acquisition system
20 comprising a charged coupled device camera adapted to capture an image of a plurality of manipulated cells in various stages of the cell cycle. The stages of the cell cycle are currently understood to include interphase, G0 phase, G1 phase, S phase, G2 phase, M phase, prophase, prometaphase, metaphase, anaphase, and telophase. The principles of the present invention specifically contemplate the application thereof on
25 additional cell cycle stages when and if they are identified.

An optical source is coupled to the image acquisition system for highlighting the plurality of manipulated cells in the various stages of the cell cycle. The illumination apparatus provides for an acquisition of the image of the plurality of manipulated cells. In a specific embodiment, the illumination apparatus has a liquid
30 light guide coupled to a light source at a remote location.

A variety of user interfaces are utile for accessing the several features of the present invention. Those having ordinary skill in the art will appreciate that different user interfaces may be required to support different research scenarios. The

present invention specifically contemplates the utilization of a wide variety of user interfaces.

Numerous benefits are achieved by way of the present invention over conventional techniques. The present invention can provide techniques for predictive
5 cellular bioinformatics that can streamline a number of important decisions made in the drug discovery industry. The present invention can be implemented using off the shelf hardware including databases. In other aspects, the present invention can find useful information about substances as well as cells or portions of cells. Furthermore, the present invention can acquire more than one feature using more than one
10 manipulation. Moreover, the present invention can provide information about a wide variety of cellular information that is not conventionally available. This information includes information about different cell components, e.g., nuclei and Golgi apparatus. Still further, the present invention provides an automated or semi-automated technique for acquiring images and populating a database. The present
15 database can be combined with others such as genomics, and the like. Moreover, the present invention can be implemented to predict, *inter alia*, a mechanism of action, toxicity, target validation, and pre-clinical disease model.

A further understanding of the nature and advantages of the invention herein may be realized by reference to the remaining sections of the specification and
20 the attached drawings.

BRIEF DESCRIPTION OF THE DRAWING

For more complete understanding of the present invention, reference is
5 made to the accompanying Drawing in the following Detailed Description of the
Invention. In the drawing:

Fig. 1 is a simplified system diagram according to an embodiment
according to the present invention;

10 Figs. 1A-1B are more detailed diagrams of database systems according
to embodiments of the present invention;

Fig. 2 is a simplified block diagram according to an alternative
embodiment according to the present invention;

Figs. 3-6 are simplified diagrams of system elements according to
embodiments of the present invention;

15 Figs. 7A-7K illustrate representative block diagrams of simplified
process steps in a particular embodiment according to the present invention;

Fig. 8A-8F illustrate representative quantified descriptors of effects of
manipulations on images of cells in a particular experiment;

20 Fig. 9 illustrates example images for different types of morphologies in
a particular experiment;

Fig. 10 illustrates a distribution of various morphologies in a cell
population responsive to drug concentration in a particular experiment;

Fig. 11 illustrates a graph of quantified features of effects of
manipulations on cells in a particular experiment;

25 Fig. 12 illustrates effects of external agents on cells in a particular
experiment;

Fig. 13 illustrates 4 panels for each marker for a plurality of A549 cells
in a particular experiment;

30 Fig. 14 illustrates 4 panels for each marker for a plurality of OVCAR-3
cells in a particular experiment;

Fig. 15 illustrates 4 panels for each marker for a plurality of OVCAR-3
cells at 20x in a particular experiment;

Fig. 16 illustrates 4 panels for each marker for a plurality of OVCAR-3 cells at 40x in a particular experiment;

Fig. 17 illustrates a representative input for a morphometric analysis program in a particular embodiment according to the present invention; and

5 Figs. 18-19 illustrate examples of the generation of pseudo-sequences and clustering in a particular embodiment according to the present invention.

Fig. 20 is a block diagram for a first research scenario;

Fig. 21 is a block diagram for a second research scenario; and

Fig. 22 is a block diagram for a third research scenario.

10 Reference numbers refer to the same or equivalent parts of the invention throughout the several figures of the Drawing.

DETAILED DESCRIPTION OF THE INVENTION

According to the present invention, techniques for determining information about manipulated cells or substances based upon living, fixed, or dead cell structures or portions of cells are provided. In an exemplary embodiment, the present invention provides a novel system including computer codes coupled to a database and user interfaces for acquiring, storing and retrieving such information. Other embodiments provide a novel image capturing system for providing digitized representations of live and dead cell structures or the like.

Fig. 1 is a simplified system diagram 10 of a cellular knowledge-based system according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The present system 10 includes a variety of elements such as a computing device 13, which is coupled to an image processor 15 and is coupled to a database 21. The image processor receives information from an image capturing device 17, which image processor and image capturing device are collectively referred to as the imaging system herein. The image capturing device obtains information from a plate 19, which includes a plurality of sites for cells. These cells can be biological cells that are living, fixed, dead, cell fractions, cells in a tissue, and the like. The computing device retrieves the information, which has been digitized, from the image processing device and stores such information into the database. A user interface device 11, which can be a personal computer, a work station, a network computer, a personal digital assistant, or the like, is coupled to the computing device.

Fig. 1A is a simplified diagram of a database system 1000 according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize many other variations, modifications, and alternatives. Database system 1000 includes a variety of techniques for processing images from biological cells, e.g., fixed, living, and dead cells, and cell portions. As shown, images are acquired 1001. These images can be from a single frame or multiple frames. As merely an example, an image processing system may analyze such images. One example of

such an image processing system is described below, but should not be construed as limiting certain claims.

In a specific embodiment, cell samples are manipulated using a compound (e.g., substance, drug). The cell samples are imaged for a simple portion or portions, e.g., manipulated cell substructure, manipulated spatial feature of cell, cell density. Image processing techniques are used to extract 1003 the feature or features from the image or images. The features can be an independent or a dependent set of cell characteristics (which may be predominately visual) including, for example, count, area, perimeter, length, breadth, fiber length, fiber breadth, shape factor, elliptical form factor, inner radius, outer radius, mean radius, equivalent radius, 10 equivalent sphere volume, equivalent prolate volume, equivalent oblate volume, equivalent sphere surface, average intensity, total intensity, optical density, radial dispersion, texture difference, and others. Each of these features corresponds to a similar manipulation by a compound. Each manipulation forms a new set of features, which are identifiable to the compound. Once each set of features has been extracted, 15 the feature set is populated 1004 into a database 1012. Accordingly, the database includes many sets of features, where each set corresponds to a different manipulation for a selected cell. Each set of features corresponding to a manipulation provides a descriptor 1009, which is also stored 1019 in the database. The descriptor is a "finger print" including each feature for the manipulation. Each descriptor may be unique, or 20 may have similarities to other descriptors or may even be the same as other descriptors for known and unknown manipulations.

The present system retrieves features, which we define as simple features herein, and forms composite features 1007 from them. More than one feature 25 can be combined in a variety of different ways to form these composite features. In particular, the composite feature can be any function or combination of a simple feature and other composite features. The function can be algebraic, logical, sinusoidal, logarithmic, linear, hyperbolic, statistical, and the like. Alternatively, more than one simple feature can be combined in a functional manner (e.g., arithmetic, algebraic). As merely an example, the composite feature equals a sum of 30 feature 1 and feature 2, where these features correspond to the same manipulation. Alternatively, the composite feature equals feature 1 divided by feature 2. Alternatively, the composite feature equals feature 1 minus feature 2. Alternatively,

the composite feature equals a constant times feature 1 plus feature 2. Of course, there are many ways that the composite feature can be defined. The present system also stores 1017 these features in the database. The composite features can also be further combined with simple features. Once these features are defined as descriptors,
5 they are stored 1019 in the database.

Fig. 1B is a simplified diagram of a database system engine 2000 according to an embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize many other variations, modifications, and alternatives. The
10 engine can be implemented into the present database for populating, searching, and predicting compound or cell characteristics. As merely an example, engine 2001 includes an input/output module 2008. The input/output module is used to input and output information from the database. The information includes, among others, a plurality of feature sets, which correspond to many manipulations. Additionally, the
15 information includes descriptors, which each corresponds to a set of features from the manipulation. The database also has a population module, which is used to configure the features based upon an entity relationship, which has been predetermined.

The database engine also has other modules. In particular, the database has a transcription module, which transfers a preselected set of features and
20 creates a descriptor from them. The transcription module can be used to take a known compound, which has features, to transcribe them into a descriptor. Alternatively, the transcription module can be used to take an unknown compound, which has features, to transcribe them into a descriptor. These descriptors are provided into the database for subsequent use. Finally, the database engine has a prediction module, which can
25 be used to potentially predict a property (e.g., mechanism of action) of an unknown compound. Here, the unknown compound is provided with a descriptor, but the property of the compound is unknown. In one embodiment, the prediction module compares a descriptor of an unknown compound with the many descriptors of known compounds, which were in the populated database. Depending upon the matching
30 criteria, the prediction module will attempt to uncover one or more descriptors of known compounds. Once the prediction module finds the descriptors of the known compounds based upon the descriptor for the unknown compound, it identifies a potential property of such unknown compound for analysis and review. Here, it is

believed that certain features of the known compound, which are similar to those features of the unknown compound may uncover a property to the unknown compound. Details of the present software engine are described more fully below.

Fig. 2 is a simplified block diagram 20 of a cellular knowledge-based system according to an alternative embodiment of the present invention. This diagram is merely an example and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Like reference numerals are used in the present diagram as the previous diagram for easy cross-referencing, but are not intended to be limiting in any manner. The present diagram 20 includes a variety of elements such as a processor 13 or computing device coupled to a database 11. The processor can be used for retrieving and storing information from the database. The system also includes a plurality of system elements, such as a cleaner 23, a dispenser 25, and an image capturing system 27, which are also coupled to the database in some embodiments. These elements can be coupled to each other through a network or the like. As merely an example, the network can be a NetWareTM network from Novell Corporation or an internet network or the Internet but can also be others and any combination thereof. The system also has an output device 31, which can be used to output information from the database, processor, or other system elements. Details of these elements are described more fully below in reference to the Figs.

Figs. 3-5 are simplified drawings of system elements according to embodiments of the present invention. These diagrams are merely examples and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. As merely an example, Fig. 3 is a simplified diagram of a processor or computing device 13. The computing device 13 includes a bus 112 which interconnects major subsystems such as a central processor 114, a system memory 116 (e.g., random access memory), an input/output ("I/O") controller 118, an external device such as a display screen 124 via a display adapter 126, a keyboard 132 and a mouse 146 via an I/O controller 118, a SCSI host adapter (not shown), and a floppy disk drive 136 operative to receive a floppy disk 138.

The computing device has other features. Storage Interface 134 may act as a storage interface to a fixed disk drive 144 or a CD-ROM player 140 operative

to receive a CD-ROM 142. Fixed disk 144 may be a part of computing device or may be separate and accessed through other interface systems. A network interface 148 may provide a direct connection to a remote server via a telephone link or to the Internet. Network interface 148 may also connect to a local area network ("LAN") or other network interconnecting many computer systems. Many other devices or subsystems (not shown) may be connected in a similar manner. Also, it is not necessary for all of the devices shown in Fig. 3 to be present to practice the present invention, as discussed below. The devices and subsystems may be interconnected in different ways from that shown in Fig. 3. The operation of a computer system such as that shown in Fig. 3 is readily known in the art and is not discussed in detail in this application. Computer code to implement the present invention, may be operably disposed or stored in computer-readable storage media such as system memory 116, fixed disk 144, CD-ROM 140, or floppy disk 138. The computer code can be organized in terms of processes or modules, depending upon the application. That is, the computer code can include a prediction module, a translation module, or other modules to carry out the functionality described herein, as well as others.

Figs. 4 and 5 are simplified diagrams of an imaging system 200 according to an embodiment of the present invention. As shown, the imaging system 200 includes a variety of features such as housing 203, which holds a stage assembly 204. The stage assembly includes an x-stage movement element 206, which is along an x-direction, and a y-stage movement element 207, which is along a y-direction. The imaging system also includes a z-direction movement element, which is perpendicular to the x-y plane. The z-direction movement motor can be attached to the stage, or to the objective nosepiece by way of the microscope housing, or as an external motor between the objective and the microscope housing. The stage can align in any one of the directions to an accuracy of one micron and less, or one-half micron and less, or one-quarter micron and less, depending upon the embodiment.

The stage holds a plate 202 or cell holder, which houses one of a plurality of samples. The plate includes a spatial array 209 of process sites. Each of the process sites can include a plurality of cells and solutions depending upon the embodiment. Each of the sites can carry a sufficient amount of solution to prevent substantial evaporation of the sample during processing in some embodiments. In embodiments for large scale analysis, the plate includes at least 96 sites, or more than

or equal to 384 sites, or more than or equal to 1,536 sites. The plate bottom is transparent and thin, which allows light to pass through the sample. Additionally, the plate is made of a suitable chemical resistant material. As merely an example, the plate can be either a 96, or 384, or 1536 or other formats from places such as Becton Dickinson of Franklin Lakes, NJ, or Corning Science Products of Corning, NY. In a preferred embodiment, the plate is a Corning Costar black-walled 96 well plate catalog #3904 from Corning Science Products of Corning, NY, but should not be limited to these in some applications, but can be others.

Also shown is the condenser for the microscope 201, which can be used to collect phase, DIC, or bright field images of the cells. Images resulting from the illumination of the samples to fluorescence, phase, DIC, or bright field techniques are collected using an image capturing device 208, which captures an image or images of cells from the plate. In a specific embodiment, the microscope is an inverted configuration with the objectives on the bottom of the plate and the condenser disposed overlying an upper surface of the sites, while the image capturing device underlies the sites. Images captured by the imaging device, whether analogue or digital, are viewed by a monitor or other devices. The image capturing device can be any camera assembly such as a charge coupled device camera, which is known as a CCD camera, or other high resolution camera capable of capturing images from the sites. In a specific embodiment, the camera is an interline CCD camera which does not require an external shutter.

In a specific embodiment, the present imaging system can be any suitable unit that is flexible for automated image collection using multi-well plastic plates. The imaging system also should be adapted to collect high-resolution images of cells on plastic or glass plates, cell growth chambers, or coverslips. The system also can be used for imaging multiple cell markers in multiple imaging conditions. To accomplish this, the microscope system has a variety of elements such as a light source, a motorized excitation filter wheel and shutter, x-y-z-motorized stage, excitation and emission filters, Fluor phase and DIC objectives, motorized objective nosepiece, dichroic filters, motorized dichroic filter cubes, phase and DIC rings and prisms, CCD camera, and software control. As merely an example, the present imaging system can have components such as those listed in the Table below.

DESCRIPTION	MAKER	MODEL
Microscope	Zeiss	100M
(x-y) motorized stage	Prior	
Xenon lamp	Sutter	Lambda
Filter wheel	Sutter	Lambda-10
Microtitre Plate holder	Prior	500-H223R
Isolation Table	Kinetic Systems	9101-24-85
Objective Spacers	Polytec PI	P-721.90
Camera	Hamamatsu	C47-95
Computer	IBM	IntelliStation
Software	Metamorph	v.4
Objectives	Zeiss	Achroplan 10x/0.25 LD-Achroplan 20x/0.4 LD-Achroplan 40x/0.6

Table: Image Acquisition System Elements

5 In a specific embodiment, the present system has the following capabilities, which are not intended to be limiting.

Image acquisition

1) Ability to automatically acquire multi-wavelength images from multiple sites on one multi-well plate, to sequentially name image files, and to log any
10 imaging parameter information with image files.

2) Ability to link images with a larger database/spreadsheet of information.

3) Ability to automatically collect multiple plates by interfacing the imaging system with a robotic arm.

15

X-Y control

1) Ability to place 96, 384, or 1536 well plates onto microscope stage and move to each well sequentially.

2) Ability to return to each well and collect another round of images (multi-site time-lapse) or ability to collect rapid time-lapse information at each well (time-lapse of many wells).

3) Ability to collect a low magnification image, automatically
5 determine features which may be of interest, automatically change the objective to a higher magnification, and collect high magnification images of a fixed number of those identified cells in the sample.

4) Ability to collect multiple frames in each site.

10 Z control

1. Ability to auto-focus with substantially minimal damage to biological specimen or fluorophore.

2. Ability to auto-focus rapidly.

15 The present embodiment of the imaging system is shown by way of Figs. 5A and 5B. These diagrams are merely examples and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The present imaging system 40 includes a variety of elements such as a microscope 41, which is preferably an epi-fluorescent microscope,
20 but can be confocal, multiphoton, or hybrid types. The microscope includes elements 41A, the motorized Z-axis; 41B, the motorized dichroic filter cube holder; and 41C, the motorized objective nosepiece. In one embodiment, the microscope is a Model 100M made by Zeiss. The microscope communicates to computer 51 through control lines 73, 75, and 76. The imaging system also has camera 50 coupled to controller
25 50A and computing device 51, which oversees and controls operations of the elements of the imaging system.

The present microscope includes drivers for spatially moving a stage in two dimensions, including an x-direction, a y-direction, and moving the objective nosepiece in a z-direction in a Cartesian coordinate system. The z-direction
30 movement is provided using a fast z-motor, which can make z-direction adjustments within a predetermined time. The z-direction movement generally provides for focussing of the sample to the camera. The focussing occurs within the predetermined time of preferably ten seconds and less, or five seconds and less, or one

second and less, depending upon the embodiment. As merely an example, the z-motor or positioner can be a model PIFOC objective nanopositioner made by a company called Physik Instrumente of Waldbronn, Germany, but also can be others. The z-motor couples to computer 51 through line 63, which may also include a controller. Depending upon the embodiment, a second z-motor 41A connected to the computer 51 by line 73 may be used to keep the z-motor 42 in the center of its travel. Alternatively, in other embodiments the stage could be provided with a z-motor allowing for movement of the stage in the z-direction.

The present stage also includes an x-y stage 43. The x-y stage moves plate 59, e.g., 96 site, 384 site, 1536 site. The x-y stage moves plate in an x-y spatial manner. The stage has an accuracy or repeatability of about 1 micron and less, or about 2 microns and less. The stage can move in a continuous manner or a stepped manner. The stage also can move up to 30 mm/sec. or faster. The stage also can move 1 mm/sec. and less, depending upon the embodiment. The stage can also step 0.1 micron and less or 1 micron and less, as well as other spatial dimensions. The stage can be one such as a Proscan Series made by Prior Scientific of Rockland, MA but can also be others. The stage is controlled via control line 61 through controller 43A, which couples to computer 51 through control line 65.

The stage includes plate holder 44. The plate holder can hold a single plate. In other embodiments, plate holder can also hold multiple plates. The plate holder can use mechanical, electrical, fluid, vacuum and other means for holding the plate or plates. The plate holder also is sufficiently stable for securing the plate. As merely an example, the plate holder is a Model 500-H223R made by Prior Scientific of Rockland, MA. In some embodiments, the plate holder may need adjustment in the z-direction to provide for a desirable focus of a sample on a plate. In these embodiments, the plate holder is supported by spacers 45 or a plurality of stage pins, which mechanically elevate the plate holder in the z-direction. These pins are generally made of a suitable material for supporting such plate holder and also are sufficiently resistant to chemicals and the like.

In some embodiments, the entire imaging system is placed on an isolation table 49. The isolation table is disposed between the microscope and support structure. The isolation table is designed to prevent excessive vibration of the plate. The isolation table is made of a suitable material such as steel and honeycomb but can

be others. The table has a thickness of about 8 inches or preferably less than about 24 inches. In one embodiment, the table is Model 9101-24-85 made by Kinetic Systems of Boston, MA.

The imaging system also has a lamp or illumination assembly 62. The lamp assembly provides for a light source (See reference letter B) to a plurality of elements in the imaging system. For easy reading, the light path is defined by the dotted lines, which are not intended to be limiting. The lamp assembly has a variety of elements such as a Xenon lamp 46. The Xenon lamp provides light at about 320 to 700 nanometers (Prefocused). The Xenon lamp is 175 or 300 Watts. As merely an example, the lamp can be a Lambda Model made by Sutter Instrument Company of Novato, CA.

Referring to Fig. 5B, the lamp assembly also has a cold mirror 58, an excitation filter wheel 48, excitation filter(s) 55, and an excitation light shutter 57. As shown, light is derived from the Xenon lamp, reflects off of the cold mirror 58, traverses through the excitation filter or filters 55, and is controlled by the excitation light shutter 57. The lamp assembly has filter wheel 48, which houses one of a plurality of filters, including excitation filters. The shutter and filter wheel are controlled via control lines 67, which are coupled to a computer 51 or other type of computing device. The control lines 67 are coupled through controller 57A (for element 57) and controller 48A (for element 48) via control line 69 to computer 51.

Preferably, light traverses from the lamp assembly through a light guide 47 to illuminate features within the plate. The light guide is suitably selected to have a flexible member, which can be used to place lamp source at a remote location away from the imaging device. The flexible member substantially keeps any vibration from the lamp assembly away from the imaging device. In some embodiments, the member is at least 1 foot away from the imaging device. The light guide is a guide, which is a flexible hose-type sleeve. The sleeve is filled with a liquid such as an aqueous solution containing chloride or phosphate. A thin layer may be formed on the inside of the sleeve. The layer can be a containing tetrafluoroethylene and hexafluoropropylene, or containing tetrafluoroethylene and perfluoromethyl vinyl ether, or tetrafluoroethylene and perfluoropropyl vinyl ether. An example of such a light guide is described in International Application No. WO/98/38537 filed February 29, 1997, and assigned to NATH, Gunther. The liquid

light guide has less than about 30% transmission loss of the light at a remote location such as the imaging system.

Light is derived from the lamp assembly and directs off of filter 56, which directs the light upward. Filter 56 can be a dichroic and emission filter, as well as others. The light traverses through microscope nosepiece 41C, and traverses through objective spacers 54. An objective 53 magnifies the light toward a predetermined point on the plate 59. The objective can be, for example, made by Zeiss of Jena, Germany, as well as other companies. The objective can be one of a plurality including 1X, 10X, 20X, 40X, and others, depending upon the application. Magnification can be further expanded or contracted by intermediate optics between the objective and the camera. Selection of filter or filters is controlled by computer 51 via control line 75.

The camera 50 captures an image of cells from plate 59. The image is obtained from light scattering off of cells or portions of cells in the plate through objective 53, through objective spacers, through filters 56, which are captured at camera 50. In this preferred embodiment, the camera is a digital camera, but can be an analogue camera. The digital camera is a CCD camera, which has 1280 by 1024 pixels, or more or less. The pixels can be 6.7 microns in dimension or more or less. The camera preferably is substantially free from an external shutter to quickly capture a plurality of images of cells from the plate. The camera is controlled via control line 71 through controller 50A, which connects to computer 51 through control line 70. The present invention can also include other types of image acquisition devices selected from at least an epifluorescence, a confocal, a total-internal reflection, a phase, a Hoffman, a bright field, a dark field, a differential interference contrast, an interference reflection, or multi-photon illumination device.

The present imaging system stores images on a high density memory device 60. The high density memory device is preferably optical, but can also be magnetic. The high density memory device can be any suitable unit that is capable of storing a plurality of images from a plurality of sites in the plate. The memory device can be a compact disk, which would generally use a compact disk burner or the like. Depending upon the embodiment, the high density memory device is used to archive the images that are captured from the camera in the imaging system. Further details

of the imaging system can be found throughout the present specification, and more particularly below.

As merely an example, the present invention can be implemented using the following sequence of steps, which have been described in a journal entry form.

- 5 Here, images are opened and objects are identified based on a background value that has been edited in starting image acquisition. Information is maintained in a spreadsheet or other database format, which has the following information for each object:

Image Name	Image Plane	Image Date and Time
Elapsed Time	Object #	Total area
Pixel area	Area	Hole area
Relative hole area	Standard area count	Perimeter
Length	Breadth	Fiber length
Fiber breadth	Shape factor	Ell. form factor
Inner radius	Outer radius	Mean radius
Average gray value	Total gray value	Optical density
Radial dispersion	Texture Difference Moment	EFA Harmonic 2, Semi-Major Axis
EFA Harmonic 2, Semi-Minor Axis	EFA Harmonic 2, Semi-Major Axis Angle	EFA Harmonic 2, Ellipse Area
EFA Harmonic 2, Axial Ratio	EFA Harmonic 3, Semi-Minor Axis	

10

After computations are done, the log file is saved. In particular, the file is saved in an appropriate place with an appropriate name.

In a specific embodiment, the present invention provides the following detailed example of journal entries, which should not limit the scope of the invention.

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Opens a DDE (Excel) File
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Move to Absolute Position)	Offset to upper left hand corner of well (1410, 1621)
Stage (Log Position)	
Stage (Scan Wells)	User picks wells to scan: runs 3x3 image collection.jnl.

3X3 IMAGE COLLECTION.jnl

Stage (Scan)	Takes 9 images of well, -1600 motor steps apart from left to right 3 columns and 3 rows, runs FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL.
--------------	--

5

FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl.

Stage (Log Position)	Logs stage position of each image
ADC – Focus	Opens up the manual focusing window with whatever focus time is current set
Show Message and Wait	Interactive: user hits enter to continue when done focusing

ADC-Acquire from Digital Camera	Takes Hoechst image
Save Using Sequential File Names	
Close	Closes image window

START IMAGE ANALYSIS.jnl

Low Pass	3x3 convolution of already opened image
Low Pass	3x3
Show Region Statistics	Interactive: Show entire image statistics. Calculate background subtraction value for step 4. by: INTENSITY Average + INTENSITY Std. Dev.
Arithmetic	Interactive: User inputs subtraction value from 3. into the constant Value field
Threshold image	Creates threshold 1 unit above 0 to 4096
Integrated Morphometry – Load State	Loads Start Image Analysis.ima Classifier 100 < area < 200000
Integrated Morphometry – Measure	Interactive: Shows area summary information about all objects. The average number is used as the Standard Area in 8.
Object Standards - Set Object Standards	Interactive: User inputs average area value from 7. into Standard Area box to be used by automated IMA for all images

IMA OBJECTS.jnl

Low Pass	3x3 convolution
Low Pass	3x3 convolution
Arithmetic	This background subtraction value needs to be manually entered into this journal from the value determined in START IMAGE ANALYSIS.jnl step 3
Threshold Image	1 unit above 0
Integrated Morphometry – Load State	Hoechst.IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Measures statistical info for all objects
Run Journal	Runs log obj and sum data.jnl

Log obj and sum data.jnl

Integrated Morphometry – Log Data	Logs object data into Sheet 1
Integrated Morphometry – Log Data	Log summary data into Sheet 2

5

COLLECT AUTOMATED IMA DATA IN ONE SPREADSHEET.jnl

Run Journal	Runs OPEN OBJECT LOG DDE FILE.JNL
Loop for all Images in a Directory	Loops IMA OBJECTS.jnl
Close Summary Log	
Close Object Log	User must manually save Excel spreadsheet

OPEN OBJECT LOG DDE FILE.jnl

Open Object Log	Opens a DDE object log into sheet 1 of an Excel spreadsheet
Open Summary Log	Opens a summary log into sheet 2

COLLECT AUTOMATED IMA DATA IN ONE SPREADSHEET 16 BIT IMAGES.jnl

Arithmetic	Interactive: Opens Arithmetic window for user to input background subtraction level from START IMAGE ANALYSIS.jnl step 3
Run Journal	Runs OPEN OBJECT LOG DDE FILE.JNL
Loop for all Images in a Directory	Interactive: Runs IMA OBJECTS 16 bit.jnl. User picks directory from which to choose.

5

IMA OBJECTS 16bit.jnl

Low Pass	3x3 convolution
Low Pass	3x3 convolution
Copy to 8-bit Image	No autoscale, to new untitled image
Save Using Sequential File Name	Saves 8bit image using previously defined Sequential File names.
Arithmetic	This background subtraction value needs to be manually entered into this journal from the value determined in START IMAGE ANALYSIS16 TO 8 BIT.jnl step 5
Threshold Image	1 unit above 0 to 255

Integrated Morphometry – Load State	Hoechst. IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Measures statistical info for all objects
Run Journal	Runs log obj and sum data.jnl

START IMAGE ANALYSIS 16 to 8 BIT.jnl

Copy to 8-bit Image	No autoscale, to new untitled image
Close	Closes 16 bit image
Low Pass	3x3 convolution
Low Pass	3x3 convolution
Show Region Statistics	Interactive: Show entire image statistics. Calculate background subtraction value for step 6. by: INTENSITY Average + INTENSITY Std. Dev.
Arithmetic	Interactive: User inputs subtraction value from 5. into the constant Value field
Threshold image	Creates threshold by 1 unit above 0 to 255
Integrated Morphometry – Load State	Loads Start Image Analysis.ima Classifier 100 < area < 200000
Integrated Morphometry – Measure	Interactive: Shows area summary information about all objects. The average number is used as the Standard Area in 10.
Object Standards - Set Object Standards	Interactive: User inputs average area value from 9. into Standard Area box to be used by automated IMA for all images

IMA OBJECTS WITH NEW LOG FILE.jnl

Run Journal	OPEN OBJECT LOG DDE FILE.JNL
Run Journal	IMA OBJECTS.jnl
Close Summary Log	
Close Object Log	User must manually save every Excel spreadsheet generated.

INTERACTIVE IMA OBJECTS.jnl

Threshold Image	User manually sets threshold
Integrated Morphometry – Load State	Hoechst. IMA Classifier 200 < area < 200000
Integrated Morphometry – Measure	Objects
Integrated Morphometry – Log Data	Into open object.log file

5

COLLECT INTERACTIVE IMA DATA.jnl

Close Object Lo g	
Open Object Log	Interactive
Annotate Log File	Interactive: experimental information that will go into the first line of the object log file
Loop for all Images in Directory	Runs INTERACTIVE IMA OBJECTS.jnl

CHANGE FILTER, COLLECT IMAGE, SAVE SEQUENTIAL FILE
NAME.jnl

Stage (Log Position)	
ADC-Focus	

Show Message and Wait	Interactive – user presses Enter when done focusing
ADC – Acquire from Digital Camera	Hoechst
Save Using Sequential File Name	
Close	Close open image.

COLLECT HOECHST AND FITC.jnl

Run Journal	FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL
Run Journal	CHANGE FILTER, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.jnl

3X3 IMAGE COLLECTION HOECHST FITC.jnl

Stage (Scan)	COLLECT HOECHST AND FITC.jnl
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5

AUTOMATED 3X3 IMAGE COLLECTION HOECHST FITC.jnl

Set Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Excel DDL files
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Move to Absolute Position)	Offset to upper left hand corner of well (1410, 1621)

Stage (Log Position)	
Stage (Scan Wells)	Interactive: user picks wells to scan: runs 3X3 IMAGE COLLECTION HOECHST FITC.jnl

AUTOMATED IMAGE COLLECTION.jnl

Sct Up Sequential File Names	Interactive: user sets up prefix name and image storage directory
Open Data Log	Opens a DDE (Excel) File
Annotate Log File	Interactive: experimental information that will go into the first line of the log file of stage positions
Stage (Go to Origin)	Origin is set as the center of well A1
Stage (Log Position)	
Stage (Scan Wells)	Interactive: user picks wells to scan: runs FOCUS, COLLECT IMAGE, SAVE SEQUENTIAL FILE NAME.JNL. Well to well travel = (-9035, -9035)

5

STARTUP.jnl

Install and Configure Devices	Open Stage Meta Devices
Set Live Video Channel	

Preferences	<u>Measure Objects</u> : Draw failed classifier objects, Exclude objects that touch the edge of the image, Enable Elliptical Fourier Parameters, turn off Warn users when measurement data will be erased <u>Image Saving</u> : Save Tiff/stk using LZW compression <u>Image Windows</u> : Use transparent thresholds.
Configure Default Paths	C:\Metamorph Data C:\Metamorph Data\Common Settings
Load Journal Taskbar	Common.JTB

Nested Journals

Automated 3x3 Image Collection

5

Loop 3x3 image collection

Loop focus, collect image, save sequential file name

Automated 3x3 image collection Hoechst FITC

10

Loop 3x3 image collection Hoechst FITC

loop Collect Hoechst and FITC

focus, collect image, save sequential file name

change filter, collect image, save sequential file name

Automated image collection

15

Loop focus, collect image, save sequential file name

Collect automated IMA data in one Spreadsheet

Open object log DDE file

Loop IMA objects

Log obj and sum data

Collect automated IMA data in one spreadsheet 16 bit images

5 Open object log DDE file

Loop IMA objects 16 bit

Log obj and sum data

Although the above has been generally described in terms of a specific
10 user interface and software code, other user interfaces and code can also be used. One
of ordinary skill in the art would recognize many other variations, alternatives, and
modifications.

Fig. 6 is a simplified diagram 600 of a cleaning and dispensing system
according to an embodiment of the present invention. This system 600 includes a
15 variety of elements such as a dispensing head 609, which is coupled to a plurality of
pipettes 601. The pipettes input and output fluids or solutions from plate 603. The
plate has a plurality of sites, each of which can be used to input cells or a combination
of cells and solution. The system also has elements to house solutions 605, which are
used to manipulate cell samples in the plate. The dispensing head is supported
20 through a support member 607, which is sufficiently rigid to allow for movement of
the head. The dispenser is coupled to the present system in a mechanical and
electrical manner, which provides for a fully integrated system for providing cell
samples to the imaging system according to the present invention.

Fig. 7A illustrates a representative block flow diagram of simplified
25 process steps of a method for determining properties of a manipulation based upon
effects of the manipulation on one or more portions of one or more cells in a
particular embodiment according to the present invention. This diagram is merely an
illustration and should not limit the scope of the claims herein. One of ordinary skill
in the art would recognize other variations, modifications, and alternatives. In step
30 700, one or more samples of cells can be provided. These cells can be live, dead, or
fixed cells, or cell fractions. The cells also can be in one of many cell cycle stages,
including G0, G1, S, G2 or M phase, M phase including the following cell cycle
stages: interphase, prophase, prometaphase, metaphase, anaphase, and telophase.

Cell components tracked in presently preferable embodiments can include proteins, protein modifications, genetically manipulated proteins, exogenous proteins, enzymatic activities, nucleic acids, lipids, carbohydrates, organic and inorganic ion concentrations, sub-cellular structures, organelles, plasma membrane, adhesion complex, ion channels, ion pumps, integral membrane proteins, cell surface receptors, G-protein coupled receptors, tyrosine kinase receptors, nuclear membrane receptors, ECM binding complexes, endocytotic machinery, exocytotic machinery, lysosomes, peroxisomes, vacuoles, mitochondria, Golgi apparatus, cytoskeletal filament network, endoplasmic reticulum, nuclear membrane, proteosome apparatus, chromatin, nucleolus, cytoplasm, cytoplasmic signaling apparatus, microbe specializations and plant specializations.

The following table illustrates some markers and cell components commonly used by embodiments according to the present invention. Other markers can be used in various embodiments without departing from the scope of the invention.

Cell component	Marker	Disease State
Plasma membrane (including overall cell shape)	Carbocyanine dyes Phosphatidylserine Various lipids Glycoproteins	Apoptosis-Cancer Apoptosis-Neural degenerative Ds
Adhesion complexes	Cadherins Integrins Occludin Gap junction ERM proteins CAMs Catenins Desmosomes	Thrombosis Metastasis Wound healing Inflammatory Ds Dermatologic Ds
Ion Channels and Pumps	Na/K Atpase Calcium channels Serotonin reuptake pump CFTR	Cystic fibrosis Depression Congestive Heart Failure Epilepsy

G coupled receptors	β adrenergic receptor Angiotensin receptor	Hypertension Heart Failure Angina
Tyrosine kinase receptors	PDGF receptor FGF receptor IGF receptor	Cancer Wound healing Angiogenesis Cerebrovascular Ds
ECM binding complexes	Dystroglycan Syndecan	Muscular Dystrophy
Endocytotic machinery	Clathrin Adaptor proteins COPs Presenilins Dynamin	Alzheimer's Ds
Exocytotic machinery	SNAREs Vesicles	Epilepsy Tetanus Systemic Inflammation Allergic Reactions
Lysosomes	Acid phosphatase Transferrin	Viral diseases
Peroxisomes/Vacuoles		Neural degenerative Ds
Mitochondria	Caspases Apoptosis inducing factor F1 ATPase Fluorescein Cyclo-oxygenase	Apoptosis Neural degenerative Ds Mitochondrial Cytopathies Inflammatory Ds
Golgi Apparatus	Lens Culinaris DiOC6 carbocyanine dye COPs	

Cytoskeletal Filament Networks	Microtubules Actin Intermediate Filaments Kinesin, dynein, myosin Microtubule associated proteins Actin binding proteins Rac/Rho Keratins	Cancer Neural degenerative Ds Inflammatory Ds Cardiovascular Ds Skin Ds
Endoplasmic Reticulum	SNARE PDI Ribosomes	Neural degenerative Ds
Nuclear Membrane	Lamins Nuclear Pore Complex	Cancer
Proteosome Apparatus	Ubiquityl transferases	Cancer
Chromatin	DNA Histone proteins Histone deacetylases Telomerases	Cancer Aging
Nucleolus	Phase markers	
Cytoplasm	Intermediary Metabolic Enzymes BRCA1	Cancer
Cytoplasmic Signaling Apparatus	Calcium Camp PKC pH	Cardiovascular Ds Migraine Apoptosis Cancer
Microbe Specializations	Flagella Cilia Cell Wall components: Chitin synthase	Infectious Ds

Plant specializations	Choloroplast Cell Wall components	Crop Protection
-----------------------	--------------------------------------	-----------------

Then, in a step 702, one or more samples of the manipulation can be provided to the cells. Manipulations can comprise one or any combination of chemical, biological, mechanical, thermal, electromagnetic, gravitational, nuclear, or temporal factors, for example. For example, manipulations could include exposure to chemical compounds, including compounds of known biological activity such as therapeutics or drugs, or also compounds of unknown biological activity. Or exposure to biologics that may or may not be used as drugs such as hormones, growth factors, antibodies, or extracellular matrix components. Or exposure to biologics such as infective materials such as viruses that may be naturally occurring viruses or viruses engineered to express exogenous genes at various levels. Bioengineered viruses are one example of manipulations via gene transfer. Other means of gene transfer are well known in the art and include but are not limited to electroporation, calcium phosphate precipitation, and lipid-based transfection. Manipulations could also include delivery of antisense polynucleotides by similar means as gene transfection. Other genetic manipulations include gene knock-outs or gene mutations. Manipulations also could include cell fusion. Physical manipulations could include exposing cells to shear stress under different rates of fluid flow, exposure of cells to different temperatures, exposure of cells to vacuum or positive pressure, or exposure of cells to sonication. Manipulations could also include applying centrifugal force. Manipulations could also include changes in gravitational force, including sub-gravitation (the preferred embodiment in outer space). Manipulations could include application of a constant or pulsed electrical current. Manipulations could also include irradiation. Manipulations could also include photobleaching which in some embodiments may include prior addition of a substance that would specifically mark areas to be photobleached by subsequent light exposure. In addition, these types of manipulations may be varied as to time of exposure, or cells could be subjected to multiple manipulations in various combinations and orders of addition. Of course, the type of manipulation used depends upon the application.

Then, in a step 704, one or more descriptors of a state in the portions of the cells in the presence of the manipulation can be determined using the images

collected on the imaging system. Descriptors can comprise scalar or vector values, representing quantities such as area, perimeter, dimensions, intensity, gray level, aspect ratios, and the like. Other types of descriptors include, but are not limited to, one or any combination of characteristics such as a cell count, an area, a perimeter, a length, a breadth, a fiber length, a fiber breadth, a shape factor, a elliptical form factor, an inner radius, an outer radius, a mean radius, an equivalent radius, an equivalent sphere volume, an equivalent prolate volume, an equivalent oblate volume, an equivalent sphere surface area, an average intensity, a total intensity, and an optical density. These descriptors can be average or standard deviation values, or frequency statistics from the descriptors collected across a population of cells. These descriptors can be further reduced using other methods such as principal component analysis and the like. In some embodiments, the descriptors include features from different cell portions or cell types. That is, a first feature can be from a nuclei and a second feature is from another cell structure such as Golgi apparatus, mitochondria, spacing between cell structures or cells themselves, as well as many others.

A presently preferable embodiment uses descriptors selected from the following table. Other descriptors can also be used without departing from the scope of the invention.

Name of Parameter	Explanation/Comments
Count	Number of objects
Area	
Perimeter	
Length	X axis
Width	Y axis
Shape Factor	Measure of roundness of an object
Height	Z axis
Radius	
Distribution of Brightness	
Radius of Dispersion	Measure of how dispersed the marker is from its centroid
Centroid location	x-y position of center of mass
Number of holes in closed objects	Derivatives of this measurement might include, for

	example, Euler number (= number of objects - number of holes)
Elliptical Fourier Analysis (EFA)	Multiple frequencies that describe the shape of a closed object
Wavelet Analysis	As in EFA, but using wavelet transform
Interobject Orientation	Polar Coordinate analysis of relative location
Distribution Interobject Distances	Including statistical characteristics
Spectral Output	Measures the wavelength spectrum of the reporter dye. Includes FRET
Optical density	Absorbance of light
Phase density	Phase shifting of light
Reflection interference	Measure of the distance of the cell membrane from the surface of the substrate
1,2 and 3 dimensional Fourier Analysis	Spatial frequency analysis of non closed objects
1,2 and 3 dimensional Wavelet Analysis	Spatial frequency analysis of non closed objects
Eccentricity	The eccentricity of the ellipse that has the same second moments as the region. A measure of object elongation.
Long axis/Short Axis Length	Another measure of object elongation.
Convex perimeter	Perimeter of the smallest convex polygon surrounding an object
Convex area	Area of the smallest convex polygon surrounding an object
Solidity	Ratio of polygon bounding box area to object area.
Extent	proportion of pixels in the bounding box that are also in the region
Granularity	
Pattern matching	Significance of similarity to reference pattern
Volume measurements	As above, but adding a z axis

Then, in a step 705, a database of cell information can be provided.

Next, in a step 706, a plurality of descriptors can be searched from a database of cell information in order to locate descriptors based upon one of the descriptors of the manipulation. Then, in a step 708, properties of the manipulation are predicted based
5 upon the properties of the located descriptors. Properties can comprise toxicity, specificity against a subset of tumors, mechanisms of chemical activity, mechanisms of biological activity, structure, adverse biological effects, biological pathways, clinical effects, cellular availability, pharmacological availability, pharmacodynamic properties, clinical uses and indications, pharmacological properties, such as
10 absorption, excretion, distribution, metabolism and the like.

In a particular embodiment, step 706 comprises determining matching descriptors in the database corresponding to a prior administration of the manipulation to the descriptors of the present administration of the manipulation. In a particular embodiment according to the present invention, combinations of
15 measurements of scalar values can provide predictive information. A database can be provided having one or more "cellular fingerprints" comprised of descriptors of cell-substance interactions of drugs having known mechanisms of action with cells. Such descriptors can be analyzed, classified, and compared using a plurality of techniques, such as statistical classification and clustering, heuristic classification techniques, a
20 technique of creating "phylogenetic trees" based on various distance measures between descriptors from various drugs. In this embodiment, numeric values for the descriptors can be used by comparison techniques. A phylogenetic tree can be created that illustrates a statistical significance of the similarity between descriptors for the drugs in the database. Because the drugs used to build the initial database are of
25 known mechanism, it can be determined whether a particular scalar value in a descriptor is statistically predictive. Finally, a compound descriptor with no known mechanism of action can be queried against the database and be statistically compared and classified among the drugs in the database that the compound most resembles.

In a particular embodiment, relationships between measured
30 morphological properties of images and physiological conditions can be determined. Relationships can include, for example, treatment of different cell lines with chemical compounds, or comparing cells from a patient with control cells, and the like. In a presently preferable embodiment, comparisons can be performed on acquired image

features. Some embodiments can comprise statistical and neural network - based approaches to perform comparisons of various features. The foregoing is provided as merely an example, and is not intended to limit the scope of the present invention. Other techniques can be included for different types of data.

5 In some embodiments, classification, clustering and other types of predictive data analysis can be performed on features extracted from cell images. In a presently preferable embodiment, statistical procedures for comparisons, classification and clustering are performed on data obtained from imaging cells.

Fragments of data preparation and pre-formatting (S language):

```
10 >tmp.frame <- Generic.Summary  
>names1 <- paste("Cell.line.5", tmp.names, sep=".")  
> by.compound.matrix <- as.matrix(arranged.by.compound)
```

Example of the code for principal component analysis (data
15 preparation) using S language:

```
all.data.princomp <- menuPrincomp(data =  
by.compound.matrix, scores = T, cor = "Correlation",  
na.action = T, print.short = T, print.importance = T,  
print.loadings = T, cutoff.loadings = 0.1,  
20 plot.screeplot = T, plot.loadings = T, plot.biplot = T,  
plot.biplot.choices = c(1,2), predict.p = F)
```

Example of clustering using a divisive hierarchical clustering
algorithm:

```
25 > div.hier.2.manhattan.cluster$call  
diana(x = tmp.sum.by.comp, diss = F, metric =  
"manhattan",  
stand = T, save.x = T, save.diss = T)
```

30 Another embodiment utilizes existing tools for biological sequence similarity searches, classification, and phylogenetic analysis. In a particular embodiment, numbers in a numerical descriptor can be substituted by one or more of nucleic acid or amino acid codes according to a one of several sets of rules. Once

converted into a corresponding nucleotide or amino acid sequence representation, the fingerprints can be analyzed and compared using software and algorithms known in the art for genetic and peptide sequence comparisons, such as GCG, a product of Genetics Computer Group, with company headquarters in Madison WI. Select
5 embodiments comprising such approaches enable the use of a broad array of sophisticated algorithms to compare, analyze, and cluster gene and protein sequences. Many programs performing this task are known to those of ordinary skill in the art, such as for example, the PHYLIP (PHYlogeny Interference Package) a package of programs for inferring phylogenies (evolutionary trees) described in (Feldenstein, J.
10 1996 Methods Enzymol 266:418-427 and Feldenstein, J. 1981 J. Mol. Evol. 17(6):368-376).

Embodiments can perform such analysis based upon factors such as numerical value, statistical properties, relationships with other values, and the like. Further details of a step of manipulation are noted more particular below.

15 Fig. 7B illustrates a representative block flow diagram of simplified process steps for determining one or more descriptors of a state in the portions of the cells in the presence of the manipulation of step 704 of Fig. 7A in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill
20 in the art would recognize other variations, modifications, and alternatives. In a step 712, an image of a cell portion is obtained. In some embodiments, the cell portion is visualized with a fluorescently labeled marker that is specific for the portion or portions of interest. A cell portion can include, for example, one or more of the following: nuclei, Golgi apparatus, and other features. The cell portion may vary in
25 select embodiments according to the invention. Then, in a step 714, a digitized representation of the image obtained in step 712 is determined. In some embodiments, steps 714 and step 712 can comprise a single step. These embodiments use a digital imaging means such as a digital camera, to obtain a digital image of the target directly. Next, in a step 716, the digital representation of the image is
30 processed to obtain image features. Image features can include such quantities as area, perimeter, dimensions, intensity, aspect ratios, and the like. Then, in a step 718 descriptors can be determined from the image features. Descriptors can comprise scalar or vector quantities and can comprise the image features themselves, as well as

composed features, such as shape factor derived by a relationship $4\pi * \text{area} / \text{perimeter}$, and the like. Descriptors can also comprise statistical quantities relating to feature characteristics across a population of cells, such as a standard deviation, and average, and the like.

5 In a preferred embodiment, cells can be placed onto a microscope, such as a Zeiss microscope, or its equivalent as known in the art. A starting point, named Site A01, is identified to the microscope. A plurality of exposure parameters can be optimized for automated image collection and analysis. The microscope can automatically move to a new well, automatically focus, collect one or more images, at
10 one or more wavelengths, move to a next well, and repeat this process for all designated wells in a multiple well plate and for multiple plates. A file having a size and an intensity distribution measurement for each color and rank for each well can then be created for the images acquired. Based on this information, a user or a computer can revisit sites of interest to collect more data, if desired, or to verify
15 automated analysis. In a presently preferred embodiment, image automatic focus and acquisition can be done using computer software controlling the internal Z-motor of the microscope. Images are taken using a 10x, 20x, or 40x air long working distance objectives. Sometimes multiple images are collected per well. Image exposure times can be optimized for each fluorescent marker and cell line. The same exposure time
20 can be used for each cell line and fluorescent marker to acquire data.

Fig. 7C illustrates a representative block flow diagram of simplified process steps for obtaining images of cell portions of step 712 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill
25 in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1). In a step 720, a sample is provided to the imaging device. Samples can be provided in 96 well plates and the like. The sample may be loaded into a microscope, such as a Zeiss microscope or equivalent.

30 (2). In a step 722, a set of optical filters is selected to shine light of the appropriate wavelength to illuminate the first sample, which may be contained in a first well designated A01.

(3). In a step 724, an automatic focusing procedure is performed for the site. In a particular embodiment, the internal z-motor of the microscope which is attached to the objective nosepiece is used for automatic focusing of the microscope. In an alternative embodiments, the plate holding the samples is moved to perform automatic focusing of the microscope, or focusing can be performed by moving optical components attached to the microscope and the like.

(4). In a step 726, images are collected for the site. Images can be collected for every color at every site. Present embodiments can provide images for up to four colors. However, embodiments are contemplated that can provide more colors by using either a monochromator coupled with excitation filters which are on a filter wheel, or by digitally separating overlapping fluorophores. Those knowledgeable in the field will know that given calibration images of single fluorophores, a look-up table can be devised which will allow for the digital removal of fluorescence bleed-through of fluorescence which may occur in optical channels other than the one for which that filter has been optimized in instances of using more than one fluorophore at once. Cell growth and density information is also collected. Cell density is determined by what percentage of the area being imaged is inhabited by cells. In some embodiments, imaging can be facilitated using one or more biosensors, molecules such as non-proteins, i.e., lipids and the like, that are luminescently tagged. However, some embodiments can also use fluorescence polarization and the like. Fluorescence polarization is a homogeneous fluorescence technology where the excited state of the molecule lasts much longer than in normal fluorescence, taking seconds to minutes to reach equilibrium, obliterating the need to wash away fluorescence markers that are not specifically bound to a marker. Further, embodiments can detect differences in spectral shifts of luminescent markers. Some fluorescence markers, such as Nile Red sold by Molecular Probes of Eugene, OR, will change its emission peak wavelength depending on its environment. One can detect these changes by monitoring the level of fluorescence at both wavelengths and reading out at ratio of the two.

(5). In a step 728, a determination is made whether more fields of view need to be taken for a particular color. If this is so, then processing continues at step 726 at a new site. Otherwise, processing continues with a decisional step 730.

Images can now be taken by repeating step 726. In a preferred embodiment 4 to 9 images are collected at each site.

(5). In a step 730, a determination is made whether more optical configurations need to be taken in order to obtain images for all differently-marked cell portions the sample. If this is so, then in a step 732 a new optical configuration is determined. Images for the new optical configuration can now be taken by repeating steps 726 and 728.

(6). In a decisional step 734, after all optical configurations and images for fields of view in a sample have been obtained, a determination is made whether any further samples remain to be analyzed. If so, a new sample is brought into view and processing continues with step 720. Otherwise, image processing is complete. In a presently preferable embodiment, image data can be stored on a CD ROM using a CD ROM burner, such as CRW4416 made by Yamaha of Japan. However, other mass storage media can also be used.

Fig. 7D illustrates a representative block flow diagram of simplified process steps for processing digitized representations of step 716 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1). In a step 740, a digitized image input is preprocessed. Preprocessing might include, but is not limited to, such operations as background subtraction, thresholding, smoothing, adoptive filtering, edge enhancements, contrast enhancements, histogram equalization. A particular combination of preprocessing steps can be applied to images in successive steps or in parallel to copies of the image.

A simplified example of a smoothing and background subtraction procedure in a MatLab language is presented in computer code below:

```
function Isubtracted = cmBackgrSubtrl(I,k)
% cmBackgrSubtrl(I,k) - simple flat background (=modal*k)
subtraction
% Y = cmBackgrSubtrl(I, k) - image Y is generated by
```



```

    % subtraction (with saturation) of modal pixel value of I
    multiplied by k
    % DEFAULT - k=1
    %
5   if (nargin == 1)
        k=1;
    end
    if (size(k)~=1)
        error('cmBackgrSubtrl: parameter k should be a number.
10  Exiting...');
    end

    %modpixnum = floor(size(I(:),1)/2);
    %sortedval = sort( double(I(:)) );
15  %modpixel = sortedval(modpixnum);
    modpixel = median(double(I(:)));
    bg = k*modpixel;

    Isubtracted = mmsubm( uint8(I), uint8(round(ones(
20  size(I))*k*modpixel )) );

```

An example of a procedure for thresholding in computer code (MatLab) is presented below:

```

function thresh = GetThreshByPerim1(I, M)
25 % GetThreshByPerim1(I) Finds optimal thresholding value
    for image I
    % N = GetThreshByPerim1(I) Finds thresholding value N for
    image I
    % N = GetThreshByPerim1(I, M) - tests threshold values up
30 to M
    % DEFAULT M = maximum pixel value in I
    % note that GetThreshByArea is significantly faster
    % finds a threshold value that causes the maximal change
    in the

```

```
% total perimeter of the objects (Russ ????)
% see Matlab_Auto_threshold1_1-23-99.doc for more details
% Note: works somewhat better on SMOOTH images (i.e.
medfilt2(I, [3 3]) two times

5
if (nargin == 0)
    error (strcat( mfilename, ' : at least one parameter
required'));
elseif (nargin == 1)
10    M = double(max(I(:)));      %test thresholds up to
maximum pixel value in I
elseif (nargin > 2)
    error (strcat (mfilename, ' : too many parameters'));
end

15
if (size(M)>1)
    error (strcat(mfilename, ' : argument M should be a
number'));
end

20
Minval = double( min(I(:)));
step = 1;

%generate vertical vector perims with total perimeters of
25 objects at different
%threshold values
for i=Minval : step : M
    bwI = im2bw(I, i/255);
    prI = bwperim(bwI);
30    pr = sum(prI(:));
    if (exist('perims', 'var') == 0) %perims is yet
undefined
        perims = pr;
    else
```

```

        perims = cat(1, perims, pr);
    end
end

```

```

5  % vector prdiffs contains differences between successive
    perimeters
    prdiffs = diff(perims);
    mindecrease = min(prdiffs);
    minvalues = find(prdiffs == mindecrease);
10 index_of_mindecrease = minvalues(1);
    thresh = index_of_mindecrease + 1;

    % =====end GetThresh1=====

```

15 Thresholding provides a specific intensity, such that pixels darker than the threshold are deemed black, and pixels lighter than the threshold are considered white. The thresholded image can be processed using binary image processing techniques in order to extract regions.

(2). In a step 742+744, the digitized image input is subjected to object
20 identification. This can be accomplished by a variety of procedures, for example by thresholding or edge detection and subsequent morphological opening and closing. Edge detection can be accomplished by means of gradient-based or zero-crossing methods, such as Sobel, Canny, Laplassian, Perwitt, and other methods.

An example of object identification procedure based on Canny edge
25 detection (in MatLab language) is presented below:

```

function Imask = cmMaskDNA1(I);
% cmMaskDNA1 - generates binary mask for cell nuclei
% through edge detection
30 % Imask = cmMaskDNA1(I)
% PARAMETERS
%   I - intensity image (grayscale)
% OUTPUT
%   Imask - BW image with objects from I

```

```

%
% For more details see Notebook Matlab_DNA_masking1_1-22-
99.doc
% Uses SDC Morphology Toolbox V0.7

5
if (nargin ~= 1)
    error('Wrong number of input parameters');
end
if (nargout ~= 1)
10    error('Wrong number of output parameters: one output
argument should be provided');
end

15 Imask = edge(I, 'canny');
Imask = mm dil(Imask, mmsecross(1));
Imask = mmero ( mmc lohole(Imask,mmsecross(1)));
Imask = mmedgeoff(Imask, mmsecross(1));
% note that mmedgeoff this command removed FILLED OBJECTS
20 but not touching OUTLINES.
% these outlines can be removed by filtering:
Imask = medfilt2(Imask, [5 5]);

%=====end cmMaskDNA1
25 =====

```

However, embodiments can also use other techniques, such as Fast Fourier Transforms (FFT) and the like as known in the art without departing from the scope of the present invention.

30 (3). In a step 746, a plurality of region features can be determined. For example, in a representative embodiment, image features can include such quantities as area, perimeter, dimensions, intensity, aspect ratios, and the like. Features not directly related to individual objects are also being extracted.

An example of a procedure for extraction of some of the features (MatLab language) is presented below:

```

function OData = cmGetObjectsData(I, Ilabel)
5  % cmGetObjectsData returns array measurements of objects
  in image "I" masked by "Ilabel"
  % EV 2-3-99; 2-10-99
  % OData = cmGetObjectsData(I, Ilabel) returns an array of
  morphological and intensity measurements
10  %    taken from a grayscale image "I". Objects are
    identified on a mask image Ilabel, usually
    %    created by bwlabel()
    % OUTPUT:
    % Each row in the output array OData represents
15  individual object
    % columns contain the following measurements:
    %
    %    1 - Index ("number" of an object);      8 -
    Solidity;
20  %    2 - X coordinate of the center of mass; 9 - Extent;
    %    3 - Y coordinate      -"-      ; 10 - Total
    Intensity;
    %    4 - Total Area (in pixels);              11 - Avg.
    Intensity;
25  %    5 - Ratio of MajorAxis/MinorAxis;        12 - Median
    Intensity;
    %    6 - Eccentricity;                        13 - Intensity of
    20% bright pixel
    %    7 - EquivDiameter;                       14 - Intensity of
30  80% bright pixel
    %
    % For details on morphological parameters see information
    on MatLab imfeature();

```

```
% Intensity parameters are either obvious or are
documented in comments in this file.

if (nargin ~= 2)
5   error ('function requires exactly 2 parameters');
end
if (nargout ~= 1)
    error ('function has 1 output argument (array X by
14) ');
10 end

% finished checking arguments

% first collect morphological parameters in a structure
15 array:
ImStats = imfeature(Ilabel, 'Area', 'Centroid',
    'MajorAxisLength',...
    'MinorAxisLength', 'Eccentricity', 'EquivDiameter',
    ...
20 'Solidity', 'Extent', 8 );

% now convert it into array (matrix) while collecting
intensity data for each object:

25 %preallocate output array:
numobjects = size(ImStats, 1);
OData = zeros(numobjects, 14);
%now convert ImStats into array and add intensity data to
it
30 for k=1:numobjects
    OData(k, 1) = k;
    OData(k, 2) = ImStats(k).Centroid(1);
    OData(k, 3) = ImStats(k).Centroid(2);
    OData(k, 4) = ImStats(k).Area;
```

```
        OData(k, 5) = (ImStats(k).MajorAxisLength) /  
        (ImStats(k).MinorAxisLength);  
        OData(k, 6) = ImStats(k).Eccentricity ;  
        OData(k, 7) = ImStats(k).EquivDiameter;  
5      OData(k, 8) = ImStats(k).Solidity;  
        OData(k, 9) = ImStats(k).Extent;  
  
        % now collect and assign intensity parameters from  
        image I  
10  
        object_pixels = find( Ilabel == k);  
        object_area = size(object_pixels, 1); %same as total  
        number of pixels in the object  
        object_intensities = double(I(object_pixels)); %  
15      need to convert to double to do math  
        sorted_intensities = sort(object_intensities); %  
        will need to get median, 20% and 80% pixels  
        total_intensity = sum(object_intensities, 1);  
        avg_intensity = total_intensity / object_area;  
20      median_intensity = sorted_intensities( floor(  
        object_area/2 ) + 1 );  
        pix20 = sorted_intensities( floor(object_area*0.2)+1  
        ) ; %brightest pixel among dimmest 20%  
        pix80 = sorted_intensities( floor(object_area*0.8)+1  
25      ) ;  
  
        OData(k, 10) = total_intensity;  
        OData(k, 11) = avg_intensity;  
        OData(k, 12) = median_intensity;  
30      OData(k, 13) = pix20; %brightest pixel among dimmest  
        20%  
        OData(k, 14) = pix80; %dimmest pixel among brightest  
        20%  
        end %for
```

```
%===== end function
cmGetObjectsData() =====
```

- 5 (4). In a step 748, quantitative descriptors, characterizing cell state are calculated based on the feature measurements extracted at step 746. For example, histogram distribution of intensities of cell nuclei provides information about the population cell cycle stages.

In a particular embodiment according to the present invention, data
 10 analysis techniques for describing the fluorescence patterns of cell portions in multiple cell lines in the presence and absence of compounds are provided. Automated image analysis techniques can include determining one or more regions from around nuclei, individual cells, organelles, and the like, called "objects" using a thresholding function. Objects that reside on the edge of an image can be included or
 15 excluded in various embodiments. An average population information about an object can be determined and recorded into a database, which can comprise a database text file or Excel spreadsheet, for example. However, embodiments can use any recording means without departing from the scope of the present invention. Values measured can be compared to the visual image. One or more types of numerical
 20 descriptors can be generated from the values. For example, descriptors such as a number of objects, an average, a standard deviation of objects, a histogram (number or percentage of objects per bin, average, standard deviation), and the like can be determined.

In a particular embodiment according to the present invention, data can
 25 be analyzed using morphometric values derived from any of a plurality of techniques commonly known in the art. For example, a software package called MetaMorph Imaging System, provided by Universal Imaging Corporation, a company with headquarters in West Chester, PA and NIH Image, provided by Scion Corporation, a company with headquarters in Frederick, Maryland.

30 Fluorescent images can be described by numerical values, such as for example, an area, a fluorescence intensity, a population count, a radial dispersion, a perimeter, a length, and the like. Further, other values can be derived from such measurements. For example, a shape factor can be derived according to a relationship

$4\pi \cdot \text{area} / \text{perimeter}$. Other values can be used in various embodiments according to the present invention. Such values can be analyzed as average values and frequency distributions from a population of individual cells.

In a particular embodiment according to the present invention,
5 techniques for the automatic identification of mitotic cells are provided. Image analysis techniques employing techniques such as multidimensional representations, frequency-based representations, multidimensional cluster analysis techniques and the like can be included in various embodiments without departing from the scope of the present invention. Techniques for performing such analyses are known in the art and
10 include those embodied in MatLab software, produced by MathWorks, a company with headquarters in Natick, MA.

Scalar values providing efficacious descriptors of cell images can be identified using the techniques of the present invention to perform predictive analysis of drug behavior. In a presently preferred embodiment, a plurality of heterogeneous
15 scalar values can be combined to provide descriptors for each manipulation. By applying predictive analysis routines to the collections of these descriptors, predictive information about any number of manipulations and cell interactions can be extracted.

Fig. 7E illustrates a representative block flow diagram of simplified process steps for analyzing image feature values to obtain descriptors of cell state of
20 step 718 of Fig. 7B in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7E illustrates an input data of descriptors of known manipulations 319. A step 320 of reformatting and transforming data 319 to
25 formats suitable for analysis is performed. Additionally, a "cleaning" process can eliminate outlying data points and the like in the data. Then, in a step 322, a decision is made whether to continue with step 324 or with step 326 based upon determining a particular type of analysis appropriate for the present application or particular type of prediction. If decisional step 322 determines processing should continue with step
30 324, then, in that step, an error estimate using a set of test descriptors is performed to estimate the quality of a prediction and processing continues with step 320. Once an optimal prediction is achieved, processing continues with step 326. In step 326, optimal transformation parameters and prediction methods are selected for use in

steps 328 and 330 which analyze data about an unknown manipulation. In a step 328, a solution is generated based upon any of techniques including training a neural network, solving a mathematical equation, applying decision tree rules and/or the like. In a step 330, an input data set of unknown descriptors 318 is reformatted and transformed based upon the optimal transformation parameters selected in step 326 using the transformation procedures in steps 320, 322 and 324. In a step 332, predictions techniques are applied to the reformatted manipulations from step 330 and the solution generated in step 328 and a plurality of properties of known manipulations 317 (e.g., therapeutic properties, and the like) in order to determine a prediction of properties of unknown manipulation 316.

Fig. 7F illustrates a representative block flow diagram of simplified process steps for a method of mapping a manipulation of cells to a physiological characteristic in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1) In a step 750, a plurality of cells, e.g., dead, live, cell fractions or mixtures of cells are provided.

(2) Then, in a step 752, the plurality of cells is manipulated, where manipulation occurs using a source(s) from one or a combination selected from an electromagnetic, electrical, chemical, thermal, gravitational, nuclear, temporal, or a biological source.

(3) Next, in a step 754, a feature value is captured from the plurality of cells. The feature value can include one or any combination of characteristics such as cell count, area, perimeter, length, breadth, fiber length, fiber breadth, shape factor, elliptical form factor, inner radius, outer radius, mean radius, equivalent radius, equivalent sphere volume, equivalent prolate volume, equivalent oblate volume, equivalent sphere surface area, average intensity, total intensity, and optical density. This list is not meant to be limiting.

(4) Then, in a step 756, a degree of presence of one or more feature values is assigned for each manipulation.

(5) In a step 758, the feature values from the plurality of cells are stored in memory locations. From the memory locations the values can be used for

statistical analyses to produce predictive information about the relatedness of the descriptors of the manipulations to one another. This information is used to infer properties of the manipulations.

Fig. 7G illustrates a representative block flow diagram of a simplified process steps for a method for populating a database with manipulated biological cell information in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. The method is generally outlined by the steps below:

(1) In a step 760, a plurality of cells in various stages of the cell cycle, A montage image that was used as a source to generate data in Appendix A is presented in Fig. 12., such as for example, the stages of interphase, prophase, metaphase, anaphase, and telophase are provided.

(2) Then, in a step 762, each of the cells in the various stages of mitotic development is manipulated.

(3) Next, in a step 764, an image of the plurality of manipulated cells is captured using image acquisition techniques in order to provide a morphometric characteristic of each of the manipulated cells.

(4) As a preferable option, in a step 766, an image database may be populated with the image of the plurality of manipulated cells.

(5) Following step 764 or optional step 766, a morphological value is calculated from the image in a step 768.

(6) In a step 770, the database is populated with the morphological value.

Fig. 7H illustrates a representative block flow diagram of simplified process steps for a method for populating a database with manipulated biological information, e.g., image acquisition parameters, image feature summary information, and well experimental parameters in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7H illustrates a step 780 in which cells are placed into site on a plate and a manipulation is applied. Then, in a step 781 an image is taken of the cells. In step 782, the image is transferred to an image archive

database. Then, in a step 783, well experimental parameters are entered into the database 787. Well experimental parameters can include cell type, manipulation and the like. In a step 784, image acquisition parameters are transferred to database 787. Image acquisition parameters can include file name, fluorophores and the like. In a
5 step 785, the image acquired in step 781 is analyzed. Then, in step 786, an image feature summary from the analysis step 785 is transferred to database 787.

In step 788, a lookup table for all analyses is provided to database 787. The lookup table provides information about the analyses. In a step 789, a query of database 787 for process data is performed. The results are reformatted. Then in a
10 step 790, the database 787 is queried. Next, in a step 791, features of the manipulations stored in the database are combined and reduced. Next, in a step 793, reduced features of step 791 can be compared. In a step 792, the results of step 793 are recorded in database 787. Then, in a step 794, a report of predictions based on comparisons performed in step 793 is generated.

15 Fig. 7I illustrates a representative block flow diagram of simplified process steps for acquiring images of manipulated biological information, e.g., cells, cell tissues, and cell substituents in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations,
20 modifications, and alternatives. Fig. 7I illustrates a step 770 in which a user sets up an image analysis procedure. Then, in a step 772, an image is read into image analysis software. Next, in a step 774, patterns and objects are identified in the image using one or more algorithms. Next, in a step 776, sets of features are extracted from the image. Then, in a step 778, feature information, descriptor values and the like are
25 exported to the database, such as database 787 of Fig. 7H, for recording. Next, in a decisional step 779, a determination is made whether any more images should be taken. If this is so, processing continues with step 772. Otherwise, image acquisition processing is completed.

Fig. 7J illustrates a representative block flow diagram of simplified
30 process steps for populating, acquiring and analyzing images of manipulated biological information in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations,

modifications, and alternatives. Fig. 7J illustrates a step 300 of placing a plate onto an imaging stage and reading a bar code. Then, in a step 301 an autofocus procedure is performed. Next, in a step 302, a first optical filter configuration is selected and an image is collected. Then, in a decisional step 303, a determination is made whether
5 more than one image per optical configuration can be taken. If so, then, in a step 304, a new position within the well is targeted and another image is collected. Then, in a decisional step 305, a determination is made whether any more images need to be collected. If this is so, step 304 is repeated until all images for a particular well have been collected. After one or more images are collected for the well, in a step 306, the
10 stage is returned to a starting position within the well, and a montage is created from collected images. The results are named with a unique file name and stored.

In a decisional step 307, a determination is made whether any more optical channels in the well can be imaged. If this is so, then in a step 308 the next optical filter configuration is selected and an image is collected. Processing then
15 continues with decisional step 303, as described above. Otherwise, if no further optical channels in the well can be imaged, then in a decisional step 309 a determination is made whether any wells remain to be imaged. If not all wells have been imaged, then in a step 310, the stage moves to the next well and processing continues with step 301, as described above. Otherwise, if all wells on the plate have
20 been imaged, then in a decisional step 311, a determination is made whether any more plates can be processed. If this is so, then processing continues with step 300 as described above. Otherwise, in a step 312, the information is stored to a CD or other storage device as a backup.

Fig. 7K illustrates a representative block flow diagram of simplified
25 process steps compound based upon information about effects of one or more known compounds on a cell population in a particular embodiment according to the present invention. This diagram is merely an illustration and should not limit the scope of the claims herein. One of ordinary skill in the art would recognize other variations, modifications, and alternatives. Fig. 7K illustrates a step 340 of populating a database
30 with descriptors for known compounds. Such descriptors can be determined from imaging the cell population. However, in some embodiments, descriptors can be derived by measurements and combinations of measurements and the like. Then, in a step 342, descriptors for the unknown compound are determined from imaging a

second cell population. The second cell population has been treated with the unknown compound. Then, in a step 344, a relationship between the descriptors determined from the unknown compound with the descriptors determined from the known compounds can be determined. Finally, in a step 346, an inference can be made about the unknown compound based upon the descriptors of the known compounds from the relationship determined in step 344.

Accordingly, the present invention provides a novel database design. In a particular embodiment according to the present invention, a method for providing a database comprises measurement of a potentially large number of features of one or more sub-cellular morphometric markers. Markers can be from any of a large variety of normal and transformed cell lines from sources such as for example, human beings, fungi, or other species. The markers can be chosen to cover many areas of cell biology, such as, for example markers comprising the cytoskeleton of a cell. The cytoskeleton is one of a plurality of components that determine a cell's architecture, or "cytoarchitecture". A cytoarchitecture comprises structures that can mediate most cellular processes, such as cell growth and division, for example. Because the cytoskeleton is a dynamic structure, it provides a constant indication of the processes occurring within the cell. The cytoarchitecture of a cell can be quantified to produce a one or more scalar values corresponding to many possible cellular markers, such as cytoskeleton, organelles, signaling molecules, adhesion molecules and the like. Such quantification can be performed in the presence and absence of drugs, peptides, proteins, anti-sense oligonucleotides, antibodies, genetic alterations and the like. Scalar values obtained from such quantification can provide information about the shape and metabolic state of the cell.

In a presently preferred embodiment, scalar values can comprise morphometric, frequency, multi-dimensional parameters and the like, extracted from one or more fluorescence images taken from a number of cellular markers from a population of cells. Two or more such scalar values extracted from a plurality of cell lines and markers grown in the same condition together comprise a unique "fingerprint" or descriptor that can be incorporated into a database. Such cellular descriptors will change in the presence of drugs, peptides, proteins, antisense oligonucleotides, antibodies or genetic alterations. Such changes can be sufficiently unique to permit a correlation to be drawn between similar descriptors. Such

correlations can predict similar properties or characteristics with regard to mechanism of action, toxicity, animal model effectiveness, clinical trial effectiveness, patient responses and the like. In a presently preferred embodiment, a database can be built from a plurality of such descriptors from different cell lines, cellular markers, and compounds having known mechanisms of action (or structure, or gene response, or toxicity).

The present invention also provides database and descriptor comparisons according to other embodiments. In a particular embodiment according to the present invention, measurement of scalar values or features can provide predictive information. A database can be provided having one or more "cellular fingerprints" comprised of descriptors of cell substance interactions of drugs having known mechanisms of action with cells. Such descriptors can be compared using a plurality of techniques, such as a technique of creating "phylogenetic trees" of a statistical similarity between the descriptors from various drugs. In a present embodiment, scalar, numeric values can be converted into a nucleotide or amino acid letter. Once converted into a corresponding nucleotide representation, the descriptors can be analyzed and compared using software and algorithms known in the art for genetic and peptide sequence comparisons, such as GCG, a product of Genetics Computer Group, with company headquarters in Madison WI. In an alternative embodiment, numeric values for the fingerprints can be used by comparison techniques. A phylogenetic tree can be created that illustrates a statistical significance of the similarity between descriptors for the drugs in the database. Because the drugs used to build the initial database are of known mechanism, it can be determined whether a particular scalar value in a descriptor is statistically predictive. Finally, a compound fingerprint with no known mechanism of action can be queried against the database and be statistically compared and classified among the drugs in the database that the compound most resembles.

In a particular embodiment, relationships between measured morphometric properties and features of images and physiological conditions can be determined. Relationships can include, for example, treatment of different cell lines with chemical compounds, or comparing cells from a patient with control cells, and the like. In a presently preferable embodiment, a clustering can be performed on acquired image descriptors. Some embodiments can comprise statistical and neural

network - based approaches to perform clustering and comparisons of various descriptors. The foregoing is provided as merely an example, and is not intended to limit the scope of the present invention. Other techniques can be included for different types of data. In some embodiments, clustering and comparing can be performed on features extracted from cell images. In a presently preferable embodiment, procedures for comparisons and phylogenetic analysis of biological sequences can be applied to data obtained from imaging cells.

Select embodiments comprising such approaches enable the use of a broad array of sophisticated algorithms to compare, analyze, and cluster gene and protein sequences. Many programs performing this task are known to those of ordinary skill in the art, such as for example, the program Phylip, available at <http://evolution.genetics.washington.edu/phylip.html>, and other packages listed at <http://evolution.genetics.washington.edu/phylip/software.html>. However, select embodiments according to the present invention can comprise a technique of statistical classification, statistical clustering, distance based clustering, linear and non-linear regression analysis, self-organizing networks, and rule-based classification.

Embodiments can perform such analysis based upon factors such as numerical value, statistical properties, relationships with other values, and the like. In a particular embodiment, numbers in a numerical descriptor can be substituted by one or more of nucleic acid or amino acid codes. Resulting "pseudo-sequences" can be subjected to analysis by a sequence comparison and clustering program.

Other types of databases can also be provided according to other embodiments. The database includes details about the properties of a plurality of standard drugs. When the descriptor of a test compound is compared to the database, predictions about the properties of the test compound can be made using any known property of the other compounds in the database. For example, properties about a compound in the database could include structure, mechanism of action, clinical side effects, toxicity, specificity, gene expression, affinity, pharmacokinetics, and the like. The descriptor of a compound of unknown structure from a natural products library could be compared to the descriptors of compounds with known structure and the structure could be deduced from such a comparison. Similarly, such information could lead to better approaches to drug discovery research including target validation

and compound analogizing, as well as pre-clinical animal modeling, clinical trial design, side effects, dose escalation, patient population and the like.

According to the present invention, databases can be integrated with and complementary to existing genomic databases. Differential genomic expression strategies can be used for drug discovery using database technology. In one particular embodiment, cell data and cellular response data can be associated with a genetic expression profile assay to form a single assay. Live cells expressing fluorescence markers can be treated with a drug, imaged and analyzed for morphometry; and then analyzed for mRNA for expression. Such embodiments can provide rapid development of tools to link cellular behavior with functional genomics.

Database methods according to the present invention can be used to predict gene function and to assist in target validation. Databases that include genetic diversity, i.e., having cellular descriptors from cells of differing genetic backgrounds (tumor, tissue specific, and gene knock out cell lines), can provide the capability to compare cells of unknown genetic background to those in the database. Similarly, the descriptor of an unknown cellular portion in the presence of multiple drugs can be queried against the descriptors of the known markers in the database. For example, if an unknown gene is tagged with Green Fluorescent Protein (GFP), the database may be used to identify the cellular portions for which that unknown gene encodes.

According to the present invention, target validation and specialized cell-based assay screening can be performed using database systems and methods to serve as a universal high-throughput cell-based assay that can evaluate the molecular mechanism of drug action. As new genes are isolated and identified, a large collection of available gene-based knowledge is becoming available. From this large collection of new genes, potential protein targets can be identified using the genomic tools of sequence analysis and expression profiling. However, unless a gene mutation is tightly linked to a disease state, further validation of individual targets is a time consuming process, becoming a bottleneck in drug discovery. Furthermore, robotics and miniaturization are making "High Throughput Screening (HTS)" the industry standard, substantially reducing the time and cost of running a target-based biochemical assay. Therefore, it is now possible to routinely screen large libraries and use a resulting "hit" to validate the target. In such approaches, a specialized cell-based assay would be developed to test hits for each target. Since this often involves

the creation of cell lines expressing new markers, this stage may also become a bottleneck that cannot keep pace with HTS. In addition, these cell-based assays may not be amenable to high-throughput screening, making it difficult to test the increasing number of analogs arising from combinatorial chemistry.

5 In a particular embodiment according to the invention, a rapid characterization of large compound libraries for potential use as pharmaceutical products can be provided by predicting properties of compounds that relate to the compounds' potential as bioactive drugs. In many drug discovery situations, virtually millions of compounds can be passed through a HTS assay against a small number of
10 validated targets. These assays produce hundreds to thousands of potential hits. These hits can then be subsequently screened by a pipeline of secondary and tertiary screens to further characterize their specificity, often time completely missing non-specific interactions with other proteins. Techniques according to the present invention can provide a replacement to such screening operations by providing
15 information about cellular accessibility and mechanism of action for the hits coming from a HTS system. Furthermore, it can replace the biochemical HTS assay and allow rapid and accurate identification of attractive compounds from large libraries without an intervening biochemical assay. The cell information can be predictive of whether to continue into an animal model for each compound, and which animal model to
20 pursue.

 The principles of the present specifically contemplate a wide variety of research methodologies, or usage scenarios, implementing these principles. The following discussion of three such scenarios is by way of illustration and not limitation. Study of the principles enumerated herein will render evident to those
25 skilled in the art certain additional methodologies or usage scenarios enabled by the teachings hereof. The present invention specifically contemplates all such modifications. The following description presents some specific embodiments and scenarios that represent a broader use of cellular phenotypic data and characterizations to deduce mechanisms of action and other features of cellular
30 responses to various stimuli. Such procedures generally involve producing a quantitative cellular phenotype based upon two or more cellular attributes and then comparing that phenotype to phenotypes previously stored and indexed. Such

procedures make use of databases or other repositories of biological information. The invention is not limited to the specific embodiments described here.

Considering first the procedure 2000 depicted in Figure 20, a compound has been identified as having a particular cellular activity. See 2004. For example, a compound may be found to inhibit the growth of certain cancer cell *in vitro* by a specific and desired mechanism of action. This may be a particular company's "gold standard."

Next, the compound is analyzed at 2006 in terms of its effect on one or more cell lines. More specifically, the compound is linked, virtually, to a particular phenotype. Two or more values or measures of cellular attributes characterize that phenotype. These attributes are quantified in the context of specific cellular markers.

In one example, the cellular marker is an organelle such as a nucleus or Golgi apparatus. Measured attributes useful for characterizing an associated phenotype include geometric parameters (e.g., size, shape, and/or location of the organelle) and composition (e.g., concentration of particular biomolecules within the organelle).

The phenotype may be characterized by administering the compound of interest to various cell lines and in various concentrations. In each example within this matrix, the attributes of interest are measured. Ultimately, certain phenotypic features (combinations of attribute values) are associated with the compound of interest. These features provide a template for the phenotype.

Next, using the phenotype as identified at 2006, the process identifies other compounds providing similar features. The goal here is to present a list of compounds having a mechanism of action similar to that of the compound that started the process. This allows researchers to identify a mechanism of action, if not already known, for their compound and to draw conclusions based upon their compound's link to other known compounds (which may not be chemically/structurally similar to the compound of interest).

Identifying similar compounds based upon phenotype can take many paths. Most will involve some mathematical basis. For example, the phenotype defined at 2006 can be represented as a fingerprint or vector comprised of multiple scalar values of cellular attributes (as described above). The phenotype representation can then be compared against known phenotypes characterized by the same format

(e.g., they are all characterized as vectors having the same attribute set, but with different values of the attributes). The comparison may be as simple as a Euclidean distance or more sophisticated as a neural network or multivariate statistical correlation.

5 The known compounds and associated phenotypes may be stored as database records or other data structures that can be queried or otherwise accessed as part of the identification procedure. The compounds may also be associated with other relevant data such as clinical toxicity, cellular toxicity, hypersensitivity, mechanism of action, etc. (when available).

10 Compounds found to be sufficiently similar to the starting compound are returned for consideration by researchers. A data processing system may rank such compounds based on degree of similarity to the starting compound. In some cases, the system may even provide similarity scores associated with the listed compounds.

15 Often researchers wish to determine whether their particular compound has clinical or biochemical effects beyond those that they are already aware of. In a typical scenario, the compound of interest was selected based upon its strong binding a target or its stimulation or inhibition of cell growth in a particular cell line. The process associated with 2010 has likely identified the compound of interest as having
20 a particular mechanism of action based on phenotypic similarity to other compounds having a similar mechanism of action. However, within the region of biochemical space, there may be subspaces (characterized by subphenotypes) that correspond to separate properties. For example, within the phenotypic space associated with one mechanism of action, there may be subspaces associated with clinical toxicity,
25 cellular toxicity (likely overlapping the clinical toxicity space), and little or no toxicity. Obviously, a researcher would like to know whether her compound is likely to be toxic.

 Thus, the process 2000 may include characterizing the compound of interest in terms of its distance from (i.e., similarity to) specific phenotypes having
30 known characteristics. In a typical example, the known characteristic is toxicity. This feature allows the researcher to quantify her compound in terms of mechanism of action AND toxicity (or in terms of two or more other relevant properties associated

with phenotype). To allow simple ranking or characterization, compounds of interest may be scored according to a simple or weighted Boolean expression.

A second scenario of interest is depicted in Figure 21. This scenario again defines a phenotype in terms of a quantifiable vector or other measure.

5 However, rather than using a compound of interest to generate the phenotype, some other cellular stimulus is used to generate the phenotype.

As shown, a process 2100 begins with receipt of cells of interest. See 2104. In many situations, the cells are produced by a genetic or epigenetic process that affects the expression level or activity of a particular protein. More generally,
10 any cellular stimulus (e.g., radiation level and type, gravity level, magnetic field, acoustic perturbations, etc.) can be used to generate the cell line of interest. Importantly, this stimulus affects the phenotype and can be correlated therewith.

In the context of drug discovery, a gene encoding for a particular target can be genetically knocked out, underexpressed, overexpressed, expressed in a non-
15 native state, etc. This may be accomplished via standard procedures involving genomic modification, translation or transcription apparatus modification (e.g., use of antisense nucleic acids), blocking target activity (using antibodies to a receptor site for example), and the like. These processes will generally affect the phenotype in some quantifiable way. Importantly, they clearly and unambiguously define a cellular
20 phenotype associated with altering the activity of the target protein.

At 2106, the process involves measuring one or more cellular features from the cell line of interest to define/quantify the phenotype. This may be accomplished as described above with reference to 2006. Next, at 2108, the cellular phenotype generated in this manner is used to identify and rank a set of compounds
25 associated with the phenotype. This operation may proceed in the manner of operations 2008 and/or 2010 from Figure 20.

Finally, at 2110, the process clusters the compounds returned at 2108 by a mechanism of action. The operation 2106 has tightly bound a mechanism of action to a phenotype. Various compounds characterized and stored in a system
30 database may be tentatively assigned a mechanism of action or may have no suggested mechanism of action. By matching their virtual phenotype to the phenotype generated at 2106, one can create or strengthen an association between the compounds and mechanism of action relevant to the stimulus at 2104.

Considering now Figure 22, a third scenario is depicted. This scenario again involves using a virtual phenotype to glean information relevant to a mechanism of action or other cellular activity. In this case, assay data from a group of compounds (e.g., a primary or focused library) is used to elucidate a phenotype.

5 As shown, a process 2200 begins by identifying a target protein. See 2204. Then, at 2206, the process involves identifying positive and negative biochemical hits. More generally, this may involve ranking a number of compounds based upon their interaction with the target. In a specific case, the compounds are ranked based upon their binding affinities to or ability to inhibit the enzymatic activity
10 of the target protein.

 After the compounds have been characterized in some manner based upon their interaction with the target, they are used to define a cellular phenotype. See 2208. Generally, the techniques to accomplish are the same as described with reference to operation 2006 of Figure 20. In this case however, one may obtain a
15 strong correlation between mechanism of action (involving the target) and phenotype by using multiple of the compounds identified at 2206. For example, some of the "best hits" may be administered to cell lines in various concentrations. And some of the least effective compounds may also be administered. Cellular attributes that are more strongly exhibited with increasing concentration of the best hits (and not
20 exhibited or exhibited only weakly upon administration of the negative hits) can be used to define the virtual phenotype. In a related approach, compounds having widely varying levels interaction with the target are administered to cells. Those cellular attributes that vary linearly or at least monotonically with the degree of interaction between the target and compound represent attributes that can be used to define the
25 virtual phenotype.

 After the cellular phenotype has been defined, previously characterized compounds may be clustered with that phenotype. See 2210. As with operation 2110 of Figure 2, this may create or strengthen an association between a mechanism of action and various compounds in a database.

30 Finally, and optionally, procedure 2200 may provide a "higher resolution" mechanism of action for the compounds identified at 2206. See 2212. Presumably interaction with the target suggests a specific mechanism of action or at least some aspect of a mechanism of action. However, a given target may participate

in a larger cellular mechanism of action – unknown to researchers. Further, a compound may that binds with the target may participate in multiple mechanisms of action – some of which do not involve the target. By linking the target (and its positive hits) to a particular phenotype, some of these additional cellular level activities can be elucidated. The defined phenotype may have been previously identified as associated with other mechanisms of action or higher resolution mechanisms of action. Thus, the phenotype identified at 2208 can be leveraged to generate a higher resolution mechanism of action at 2212.

As suggested in the above discussion, compounds and associated phenotypes may be stored as database records. Such databases can take on many flavors. In one example, a database includes various pieces of information relevant to oncology. Such database may include numerous compounds classified by cellular phenotype, mechanism of action, toxicity, etc. More specifically, the database may include data on commercially available compounds clustered by cellular phenotypes corresponding to mechanisms of action. Further the databases of interest may extended or combined (via standard relational tables and algebra for example) to include additional data such as pharmacology data, cellular genomics data, gene expression data, protein expression data, etc. In a specific example, the database includes measurements made on a subset of the NCI60 cell lines, using DNA, Golgi apparatus, and/or microtubules as markers for defining the phenotypes. Other data includes dosage response information, variation in effect over time, etc. The compounds populating the database could include known National Cancer Institute oncology study compounds. In a specific embodiment, the compound set includes some or all of the compounds mentioned in the article “A gene expression database for the molecular pharmacology of cancer,” Nature Genetics, 24, pp. 236-244 (March 2000).

Various biological analyses may be conducted to develop additional information for characterizing compound mechanisms of action, etc. For example, a cell count analysis may be used to develop dose response curves, GI 50 data, etc. The cell cycle may also be analyzed to find out how various stages in the cycle vary in response to particular stimuli. The Golgi apparatus may be analyzed to determine whether it is in a normal state, a dispersed state, a diffused state, etc. As another example, tubulin may be analyzed to determine whether it is normal, de-polymerized,

over-polymerized, bundled, etc. Obviously, combinations of such analyses may be performed. For example, properties of the Golgi apparatus or tubulin may be analyzed over one or more cell cycles.

In some embodiments, techniques according to the present invention can provide tools for the later stages of drug development such as clinical trial design and patient management. The properties of known drugs, such as clinical trial and patient response information, will be used in a similar fashion as the pre-clinical information to provide predictions about the properties of novel compounds. Because the human cell is the locus of drug action, a database containing drug-cell interactions will be able to provide predictive value for this aspect of drug development.

Although the above has generally been described in terms of specific hardware, software, and methods, it is understood that many alternatives can exist. In particular, the present invention is not limited to a particular kind of data about a cell, but can be applied to virtually any cellular data where an understanding about the workings of the cell is desired. Thus, in some embodiments, the techniques of the present invention could provide information about many different types or groups of cells, substances, and genetic processes of all kinds. Of course, one of ordinary skill in the art would recognize other variations, modifications, and alternatives. Some examples according to the present invention are provided below.

EXPERIMENTS

To prove the principle and demonstrate the objects of the present invention, experiments have been performed to determine the effects of manipulations on cell structure using imaging and analysis techniques applied to a variety of situations. These experiments were performed by growing multiple cell lines in the presence of multiple compounds, or substances. Cells were fixed and stained with fluorescent antibodies or labels to multiple cellular portions. One or more images of the cells were then obtained using a digital camera. Descriptors were built by quantifying and/or qualifying patterns of one or more feature from each image in the cell lines under study. A database was built from the descriptors. As the database grows, it should be able to predict the mechanism of action of an unknown drug by comparing its effect with the effects of known compounds or to identify data clusters within large libraries of compounds.

In a first experiment, an automated method to count the number of cells and differentiate normal, mitotic, and apoptotic cells was created.

Approximately, 5,000 HeLa cells were plated per well in a 96 well plate and grown for 3.5 days. The cells were fixed with -20° MEOH for 5 minutes, washed with TBS for 15 minutes, and then incubated in 5 mg/ml Hoechst 33342 in TBS for 15 minutes. Then, 72 images were collected with a 40x objective and 75 ms exposure time.

The analysis was performed on objects that met a certain size criteria that was based on 1) measuring the size of objects in the image that were clearly not cells and 2) excluding the first peak of the area histogram (Fig. 8B values 1-4654).

Histograms of the individual object data were generated for each type of feature. Fig. 8A shows the histogram for average intensity, and Fig. 8B shows histogram data for the area of each object. Fig. 8C shows the scatter plot of the average intensity vs. the area of all of the objects. The pattern of the scatter plot showed an interesting pattern: a large cluster of cells in one region of the graph, with a scattering of object points in other regions. Because mitotic structures are identified as particularly bright objects, most likely due to the biological fact that the chromatin is condensed, the original Hoechst images could be used to identify which cells were either undergoing mitosis, or otherwise looked abnormal. Manual inspection of 917 cells resulted in the classification of each object. Fig. 8D shows a graph where each type of cellular classification is delimited. This graph clearly shows that the mitotic nuclei are brighter than the interphase nuclei. Further, the different phases of the cell cycle can be separated using these two features. Figs. 8E-8F show bar graphs of the average and standard deviations of the areas and average intensities for each cell classification type. These graphs show that interphase nuclei are statistically less bright than mitotic nuclei and that telophase nuclei are statistically smaller than other mitotic nuclei.

Each image was thresholded to an intensity level of 20. A standard area value was set at 9500 pixels. Automated information gathering about all of the objects was done and collected into an Excel spreadsheet (for more information see, section on imaging system). The following information was recorded:

IMAGE NAME
OBJECT #

AREA
STANDARD AREA COUNT
PERIMETER
FIBER LENGTH
FIBER BREADTH
SHAPE FACTOR
ELL. FORM FACTOR
INNER RADIUS
OUTER RADIUS
MEAN RADIUS
AVERAGE INTENSITY
TOTAL INTENSITY
OPTICAL DENSITY
RADIAL DISPERSION
TEXTURE DIFFERENCE MOMENT
EFA HARMONIC 2, SEMI-MAJOR AXIS
EFA HARMONIC 2, SEMI-MINOR AXIS
EFA HARMONIC 2, SEMI-MAJOR AXIS
ANGLE
EFA HARMONIC 2, ELLIPSE AREA
EFA HARMONIC 2, AXIAL RATIO
EFA HARMONIC 3, SEMI-MINOR AXIS

The following results were obtained:

- 1,250 objects were counted
- 201 of those objects has standard area counts > 2 (area > 19000 pixels)
- 195 objects had areas < 6000 pixels
- 1529 objects estimated in total
- 1328 object areas are > 6000 pixels
- The data was reduced to 917 objects that were $6000 < \text{area} < 19000$
- For the 917 objects a scatter plot of area vs. average intensity and a histogram of the average intensity were generated.

- 116 objects that had average intensity intensities > 60 were manually looked at to determine their morphology.

- Of those 116 objects:

6 were dead or indistinguishable

5 4 were interphase

30 were prophase

32 were metaphase

24 were anaphase

20 were telophase (10 pairs)

10

- 12 prophase objects were missed because of gray scale cut off. (8 of those prophase cells had gray scale values > 57 , as did 7 interphase)
- 1 telophase object was missed because it was too small (< 6000)
- 1 prophase object was missed because it was too big (> 1900)
- 16 mitotic objects were missed because they were parts of objects with standard count > 2 .

15

In sum, out of 917 single objects, the analysis correctly identified 106 out of 130 mitotic objects, or (81% predictive, 91% of identified mitotics). Out of 917 single objects, the analysis incorrectly identified only 10 non-mitotics as mitotics (1% total, 8% of identified mitotics); 14 mitotics as interphase (1.4% total, 1% interphase). An automated classification system that would automatically assign values to each object using these or other measurement features can thus be developed, utilizing the principles set forth herein.

In a second experiment, the effects of Taxol on MDCK cells and the different types of morphological effects were observed. A plurality of MDCK cells grown in 96 well plates were treated with Taxol for 4.5 hours at different concentrations (10 uM-1pM). They were then fixed, labeled with Hoechst, and imaged.

This experiment used a labeling protocol comprising: MEOH fix at – 20°, Wash in PBS, Block in PBS/BSA/Serum/Triton-X 100, Incubate with 5 µg/ml Hoechst 10 minutes, and wash.

Cells were inspected for different morphologies and manually counted at each different drug concentration in one well. Fig. 9 shows example images from each drug concentration and the different types of morphologies and cells are highlighted. Fig. 10 shows the distribution of each morphology within the cell population as a function of drug concentration. The higher the concentration of Taxol, the larger proportion of cells underwent apoptosis, and the fewer number of normal mitotic cells were detected.

In a third experiment, the purpose was to determine whether the automated analysis methods developed in the first experiment can detect differences in Hoechst morphology in the presence of 6 known compounds at one concentration and exposure time in one cell line. In this experiment, HeLa cells were separately treated with 6 compounds with known mechanism of action. The quantitative methods described in the first experiment were applied to the Hoechst images.

Approximately 5,000 HeLa cells per well were plated in a Costar black-walled 96 well tissue culture treated plate and left to recover in the incubator for 24 hours. After this time, 10 ug/mL of cytochalasin D (CD), Taxol, hydroxyurea, vinblastine, nocodazole, and staurosporine was added to different wells at a 1:100 addition in DMSO.

The cells were incubated in the presence of drug for 24 more hours. After 24 hours, the cells were removed and fixed as in the first experiment. Then, 9 images per well were collected of the Hoechst staining using a 10x objective.

The low magnification images taken of Hoechst were run through the automated image analysis method described in the first experiment. Plots of the average intensity and area were made of each compound. Fig. 11 shows the scatter plots of the compounds. The scatter plots of each compound are visually distinct. For example, cells treated with CD are smaller than control, and cells treated with Hydroxyurea are larger and brighter. Furthermore, the number of cells per well was very different (data not shown).

The effects of different compounds can be clearly and automatically distinguished by identifying changes in cellular morphology. This method can also be used to count adherent cells.

The next experiment was to develop clustering algorithms that assign statistically meaningful values to the representative two dimensional data shown in Fig. 10, and even more complicated clustering of all of the multidimensional data that can be extracted across one, and multiple images.

A fourth experiment was performed to obtain high magnification images of two markers in the presence of drugs. In this experiment, HeLa cells were treated with 80 generic compounds with known mechanism of action. The quantitative methods described in the first experiment were applied to the Hoechst images.

Approximately 5,000 HeLa cells per well were plated in a Costar black walled 96 well tissue culture-treated plate and left to recover in the incubator for 24 hours. After this time, 10 ug/mL of each compound from the Killer Plate from Microsource Discovery Systems (Gaylordsville, CT) was added to different wells at a 1:100 addition in DMSO. The cells were incubated in the presence of drug for 24 more hours. After 24 hours, the cells were removed and fixed as in the first experiment. In addition to being labeled with Hoechst 33342 (against chromatin), cells were also labeled with 1 unit of rhodamine-conjugated phalloidin (against actin) for 30 minutes.

The 96 well plate was imaged twice. Once, 9 images per well were collected of the Hoechst staining using a 10x objective. After this, one image per well of both the phalloidin and Hoechst staining was collected using a 40x objective.

The resulting high magnification images were analyzed qualitatively and distinct pattern differences were detected in both the Hoechst and phalloidin images. Fig. 12 shows three example images from the experiment. The top row is the Hoechst staining, and the bottom row is the phalloidin staining from the same well. The columns show the images from wells treated with just DMSO (control), cytochalasin D, and Colchicine. The morphology of each marker is different in the presence of each drug. Interestingly, there is an effect in the morphology of the chromatin in the Hoechst image of cytochalasin D, which directly targets the actin cytoskeleton (and thus there is an expected effect in the phalloidin image). Also, there is an effect on the actin cytoskeleton, compared to control, in the presence of colchicine that directly targets the microtubule network.

The low magnification images were analyzed as described in the first experiment, and different patterns were seen in both the average intensity vs. area plots, and in the number of cells per well (data not shown). Thus, changes in patterns of a marker that is "down-stream" from the direct target of a compound are detectable. Automated image analysis protocols for actin and other markers can be developed similarly, again utilizing the principles set forth herein.

A fifth experiment was performed to test quadruple labeling of 9 different cell lines grown in normal conditions. In this experiment, NCI-H460, A549, MDA-MD-231, MCF-7, SK-OV-3, OVCAR-3, A498, U-2 OS, and HeLa cells were plated. Then, the cells were fixed and stained for portions of the each cell known as DNA, tubulin, actin, and Golgi.

The following table summarizes the procedures for this experiment:

Action	Active Ingredient/Notes	Buffer	Vol/ well	Desired Time	Temp
Remove media	NOTE: gently by pipetting, not aspiration				
Fix	4% Formaldehyde	PBS	100μl	20 min	rt
Wash		TBS	100μl	5 min	rt
Wash		TBS	100μl	5 min	rt

Permeablize	0.1% Triton X-100	TBS	100 μ l	10 min	rt
Permeablize	0.1% Triton X-100	TBS	100 μ l	10 min	rt
Block	% BSA % Serum Filter sterilize before use	TBS w/azide	100 μ l	1hr or o/n	rt or 4°C
Primary Antibody	1:1000 dilution of DM1 α	TBS + 1% BSA + 0.1% TX-100	50 μ l	1hr or o/n	rt or 4°C
Wash		TBS	100 μ l	5 min	rt
Wash		TBS	100 μ l	5 min	rt
Wash		TBS	100 μ l	5 min	rt
Fluorescent Stain	FITC lens culinaris 1:500 Rhodamine-Phalloidin 1:500 CY5 goat anti-mouse 1:100	TBS + 1% BSA + 0.1% TX-100	50 μ l	1 hr.	rt, dark
Wash		PBS	100 μ l	5 min	rt, dark
Hoechst	1:1000 dilution of 5mg/ml	TBS	100 μ l	15 min	rt, dark
Wash		PBS	100 μ l	5 min	rt, dark
Wash		PBS	100 μ l	5 min	rt, dark
Wash		PBS	100 μ l	5 min	rt, dark
Store		PBS	200 μ l	1 month	4°C

Cells were plated out at different densities for 48 hours. Cells were fixed and labeled by the above method. Cells were imaged using an automated imaging system that collected 9 images from each marker using a 10x objective.

Higher magnification images were collected of a few cells for demonstration purposes.

In this experiment, each cell line demonstrated different morphological patterns as determined by phase. For example, A549 cells are much more compacted than OVCAR-3 cells as determined by phase contract imaging (data not shown). The different fluorescent markers showed even bigger differences between different cell lines. Figs. 13 and 14 show 4 panels of each marker for A549 (Fig. 13) and OVCAR-3 cells (Fig. 14). The markers are Hoechst (upper left), Phalloidin (upper right), Lens culinaris (lower left), and DM1a antibody (lower right). The following table summarizes the qualitative differences between these images:

MARKER	A549	OVCAR3
Hoechst/DNA	small	large
Phalloidin/actin	fuzzy	crisp - many stress fibers
Lens culinaris Golgi	compact	Disperse/punctate
DM1alpha Tubulin	perinuclear	evenly distributed

Higher magnification images were taken of the OVCAR3 cells. Fig. 15 shows the same markers at 20x, and Fig. 16 shows the markers at 40x. While the highest magnification images show the most detail, these images illustrate that very little morphological or feature information is lost in the 10x images.

These data exemplify the differences in morphology seen between different cell types. Thus the automated image analysis software can be customized for each marker in each cell type. Different drugs should effect these morphologies differentially.

An automated quantification method for each marker and cell line can be similarly developed.

A sixth experiment was conducted with a more sophisticated software package and to develop more flexible image recognition algorithms. In this experiment, prototype image features extraction was performed using MatLab programming language with image toolbox and SDC morphology toolboxes. Algorithms are being developed that will automatically identify objects on images and

to measure various morphological and feature parameters of these objects. Many different features for each of the cellular markers were acquired.

An example of a MatLab program called "AnalyseDNA" that takes as an input an unlimited number of images, identifies individual objects in these images based on either their intensities, or based on edge-detection algorithms, and extracts a number of morphological and intensity characteristics of these objects. A copy of this program follows:

Listing of the AnalyseDNA.m program and of some of the
supporting subroutines

10

```
function files_analysed = AnalyseDNA(filemask, outpath,
nx, ny, filter_range, dext, modifier, sfname)
% AnalyseDNA performs measurements on files of DNA images
% V1. EV 2-11-99; 2-15-99; 2-16-99
```

15

```
%
% files_analysed = AnalyseDNA(filemask, outpath, nx, ny,
filter_range, dext, modifier, sfname)
%
% PARAMETERS:
```

20

```
% ALL PARAMETERS ARE OPTIONAL
```

```
%
```

```
% FILEMASK - mask for file names to be analyzed
INCLUDING PATH(for example c:\images\*.tif)
```

25

```
% DEFAULT '.*.tif' (all *.tif files in the current
directory).
```

```
%
```

```
% OUTPATH - path to a directory where all the output
files will be placed.
```

```
% DEFAULT - output is saved in the same directory
```

30

```
which contains images
```

```
%
```

```
% NX, NY - number of individual images in montage
images along X and Y axes (DEFAULT 1)
```

```
%
```

```
%    FILTER_RANGE - 3 col-wide array (or[]). Specifies
how data is filtered when summary is calculated
%    this parameter internally is passed to GetDNADData
and then to GetSummaryData - see these
5 %    functions for details. For example: [2 2 Inf; 6 100
8000] will case all raws of data for which
%    values in column 2 are less than 2 and all raws
where values in column 6 are less than 100 or
%    more than 8000 to be excluded from all
10 calculations of a summary.
%    DEFAULT - [] (means do not filter, summarize all
data)
%
%    DEXT - string. Extension for data files being saved.
15 %    DEFAULT 'dat';
%
%    MODIFIER - this modifier is 'SUMMARY', summary file
is created;
%    'SUMMARY ONLY' - only summary is generated,
20 data for individual files are not saved
%
%    sfname - string. File name of a summary file
%    DEFAULT 'summary[date].dat'
%
25 % OUTPUT:
%
%    AnalyseDNA works on image files or montages. For
each image file it creates a tab-delimits file of
measured
30 %    parameters of all the objects in the montage with
the same base name as a montage file and extension
specified
```

```
%      by dext parameter (or .dat by default) and file
'errors[date].err' - with the list of files that matched
the
%      filemask but could not be processed.
5 %      If 'summary' or 'summary only' modifier is
specified, it also creates a single file
'summary[date].dat' (or
%      different extension, if specified by DEXT) which
contains summary information for all analyzed files.
10 %
%      ALL OUTPUT FILES are saved in a directory specified
by OUTPATH parameter
%
%      RETURNS *files_analysed* - number of files that have
15 been successfully processed.
%
%      Column designations in the output files are
described in GetDNADData
%
20 % FILE NAME CONVENTIONS
%      AnalyseDNA attempts to identify a number for each
file to identify the file in summary output.
%      It does that by looking for the first space or
underscore, followed by a number and then takes
25 %      as many successive numbers as it can find. If it
fails to identify a number it assigns a
%      default which is -1
%
%
30 % SEE ALSO GetDNADData, GetSummaryData
%
% TO DO      improve error handling in opening and writing
files (GLOBAL error_file ?)
```

```
%          include procedures for writing text headers
into the output files

if nargin > 8
5   error ('Wrong number of input parameters');
end
if nargout >1
    error ('Wrong number of output parameters: only one
allowed');
10 end

% set defaults
need_summary = 0;
summary_only = 0;
15 use_default_outpath = 0;
datestring = datestr(floor(now));
if nargin == 7      % set default summary file name
    sfname = ['summary' deblank(datestring)]; % extension
will be appended later based on dext
20   if deblank(upper(modifier)) == 'SUMMARY'
        need_summary = 1;
    elseif deblank(upper(modifier)) == 'SUMMARY ONLY'
        need_summary = 1;
        summary_only = 1;
25   else
        error (['Wrong parameter: unknown modifier '
modifier]);
    end
end
30
if nargin == 5
    % default data file extension
    set_dext = 'dat';
end
```

```
    if nargin == 4
        % default filter range
        filter_range = [];
    end
5   if nargin == 3
        ny = 1; % default number of images in montage along Y
    end
    if nargin == 2
        nx = 1;
10  end
    if nargin == 1
        use_default_outpath = 1;
    end
    if nargin == 0
15  filemask = '*.tif'
    end

    % check parameters
    if ( ~ischar(filemask) | ~ischar(dext) | ~ischar(sfname)
20  )
        error('Wrong parameter type: filename, filepath,
dext and sfname should be strings');
    end
    if ( ( size(nx) ~= [1 1] ) | ( size(ny) ~= [1 1] ) )
25  error ('Wrong parameter type: nx and ny should be
scalars (1x1 arrays)');
    end
    if (~isempty(filter_range) & size(filter_range, 2) ~= 3)
        error ('Wrong parameter type: filter range should be
30  [] or 3 - cols-wide array');
    end
    % end testing parameters

    % Generate list of files to process
```

```
datapath = getpath(filemask);
if use_default_outpath == 1
    outpath = datapath;
5  end
if exist(outpath, 'dir') ~= 7
    error(['Path ' outpath, 'not found. Exiting..']);
elseif exist(datapath, 'dir') ~= 7
    error(['Path ' datapath, 'not found. Exiting..']);
10 end

sfname = makefullname(outpath, sfname, dext);
if need_summary == 1
    if exist(sfname, 'file')
15         disp(['File ', sfname, 'already exists!']);
        input ('Press ^C to abort, Enter to delete and
continue');
        delete(sfname);
    end
20 end

flist = FileList(getfname(filemask), datapath);
numfiles = size(flist, 1); % total number of files to
25 process
disp(['About to process ', num2str(numfiles), ' files']);
%DEBUG - commented out "input" to run from Wrod
input('Press ^C to abort, Enter to continue');

30 % main loop where the job gets done:
error_file = makefullname(outpath, ['error' datestring
'.err']);
num_processed = 0;
num_error = 0;
```

```
for i = 1:numfiles
    % first generate file name for a data output file
    current_fullname = flist(i, :); % full name with path
    and extension
5    current_datafile = makefullname(outpath,
    makefname(getbasefname(current_fullname), dext) );

    %extract number from a filename
    fnumber = getfilenumber(current_fullname);
10
    % load an imagefile, record errors
    read_error = 0;
    try
        I = imread(current_fullname);
15        %DEBUG
        disp(['Image file #', num2str(fnumber), '
loaded']);
    catch
        % record file-opening error in an error_file
20        read_error = 1;
        num_error = num_error +1;
        msg = [current_fullname ': ' lasterr];
        add_error_msg(error_file, msg);
    end
25
    % extract and write data to a file in outpath
    if read_error ~=1
        if (need_summary == 0)
            %DEBUG
30            disp(['Starting analysis of file #',
num2str(fnumber), '.']);
            current_data = GetDNADData(I, nx, ny, fnumber);
            %DEBUG
```

```

        disp (['Finished analysis of file #',
num2str(fnumber), '.']);
        %load current_data.mat 'current_data';
        write_data(current_data, current_datafile);
5      else      %summary needed
        %DEBUG
        [current_data, current_summary] = GetDNADData(I,
nx, ny, fnumber, filter_range);
        %load current_data.mat 'current_data';
10      %load current_summary.mat 'current_summary';
        write_summary (current_summary, sfname);
        if summary_only ~= 1
            write_data(current_data, current_datafile);
        end
15      end
    end
end % of the main for loop
num_processed = numfiles - num_error;

20  %=====end function AnalyseDNA()
=====

%=====
=====

25  function result = add_error_msg(filename, msg)
    % adds string MSG to an errorfile FILENAME
    % returns 1 if success, 0 if failure

    err_FID = fopen(filename, 'at');
30  if err_FID == -1
        warning(['Can not open error file ' filename]);
    else
        fprintf(err_FID, '%s\n', msg);
        fclose(err_FID);

```



```

end
%=====end function add_error_masg()
=====

5  %=====
=====
function N = getfilenumber(fname)
% returns the first number extracted from a file name
(string) or -1 if fails to extract any number
10 numbers = NumbersFromString( getfname(fname) ); % vector
of all numbers encoded in the name

                                % (but not in the path, even if
present)
15 if isempty(numbers)
    N = (-1); % return -1 if no numbers found in the
name
else
    N = numbers(1);
20 end

%===== end function getfilenumber()
=====

25 %=====
=====
function result = write_data(data_array, file_name)
% writes data in a data_array in a tab-delimited ascii
file.
30 % result is 0 if success and -1 if failure
% if file_name exists, overwrites it
result = -1;
try
    fid = fopen(file_name, 'wt');

```

```

        if fid ~= -1
            for k = 1:size(data_array, 1)
                fprintf(fid, '%g\t', data_array(k, :));
                fprintf (fid, '\n');
5         end
        test = fclose(fid);
        result = -1;
    catch
        result = -1;
10    end

%===== end function write_data()
=====

15 %=====
=====
function result = write_summary (s_vector, file_name)
% appends summary vector s_vector to a file_name (ASCII
tab-delimited file).
20 % if file_name does not exist, creates it.
% result is 0 if success and -1 if failure
%
result = -1;
try
25     % debug
        fid = fopen(file_name, 'at');
        result = fprintf(fid, '%g\t', s_vector);
        result = fprintf(fid, '\n');
        result = fclose(fid);
30     result = 0;
    catch
        result = -1;
    end

```

```

% ===== end function write_summary()
=====

function Data = GetObjectsData(I, Ilabel)
5 % GetObjectsData returns array measurements of objects in
  image "I" masked by "Ilabel"
  % EV 2-3-99; 2-10-99
  % OData = GetObjectsData(I, Ilabel) returns an array of
  morphological and intensity measurements
10 %   taken from a grayscale image "I". Objects are
  identified on a mask image Ilabel, usually
  %   created by bwlabel()
  % OUTPUT:
  % Each row in the output array OData represents
15 individual object
  % columns contain the following measurements:
  %
  %   1 - Index ("number" of an object);      8 -
  Solidity;
20 %   2 - X coordinate of the center of mass; 9 - Extent;
  %   3 - Y coordinate      -"-      ; 10 - Total
  Intensity;
  %   4 - Total Area (in pixels);              11 - Avg.
  Intensity;
25 %   5 - Ratio of MajorAxis/MinorAxis;        12 - Median
  Intensity;
  %   6 - Eccentricity;                        13 - Intensity of
  20% bright pixel
  %   7 - EquivDiameter;                       14 - Intensity of
30 80% bright pixel
  %
  % For details on morphological parameters see information
  on MatLab imfeature();

```

```
% Intensity parameters are either obvious or are
documented in comments in this file.
% Procedures in this file are documented in notebook file
"MATLAB Measuring Nuclei (1) 1-29-98.doc"

5
if (nargin ~= 2)
    error ('function requires exactly 2 parameters');
end
if (nargout ~= 1)
10    error ('function has 1 output argument (array X by
    14) ');
end

% finished checking arguments

15
% first collect morphological parameters in a structure
array:
ImStats = imfeature(Ilabel, 'Area', 'Centroid',
    'MajorAxisLength', ...
20    'MinorAxisLength', 'Eccentricity', 'EquivDiameter',
    ...
    'Solidity', 'Extent', 8 );

% now convert it into array (matrix) while collecting
25 intensity data for each object:

%preallocate output array:
numobjects = size(ImStats, 1);
OData = zeros(numobjects, 14);
30 %now convert ImStats into array and add intensity data to
it
for k=1:numobjects
    OData(k, 1) = k;
    OData(k, 2) = ImStats(k).Centroid(1);
```

```
    OData(k, 3) = ImStats(k).Centroid(2);
    OData(k, 4) = ImStats(k).Area;
    OData(k, 5) = (ImStats(k).MajorAxisLength) /
    (ImStats(k).MinorAxisLength);
5    OData(k, 6) = ImStats(k).Eccentricity ;
    OData(k, 7) = ImStats(k).EquivDiameter;
    OData(k, 8) = ImStats(k).Solidity;
    OData(k, 9) = ImStats(k).Extent;

10    % now collect and assign intensity parameters from
    image I

    object_pixels = find( Ilabel == k);
    object_area = size(object_pixels, 1); %same as total
15    number of pixels in the object
    object_intensities = double(I(object_pixels)); %
    need to convert to double to do math
    sorted_intensities = sort(object_intensities); %
    will need to get median, 20% and 80% pixels
20    total_intensity = sum(object_intensities, 1);
    avg_intensity = total_intensity / object_area;
    median_intensity = sorted_intensities( floor(
    object_area/2 ) + 1 );
    pix20 = sorted_intensities( floor(object_area*0.2)+1
25    ) ; %brightest pixel among dimmest 20%
    pix80 = sorted_intensities( floor(object_area*0.8)+1
    ) ;

    OData(k, 10) = total_intensity;
30    OData(k, 11) = avg_intensity;
    OData(k, 12) = median_intensity;
    OData(k, 13) = pix20; %brightest pixel among dimmest
    20%
```

```

        OData(k, 14) = pix80; %dimmest pixel among brightest
        20%
    end %for

5   %===== end function
    GetObjectsData()=====

    function Imask = MaskDNA1(I);
10  % MaskDNA1 - generates binary mask for cell nuclei
    through edge detection
    % EV 1-22-99; 2-6-99; 2-10-99
    % Imask = MaskDNA1(I)
    % PARAMETERS
15  %   I - intensity image (grayscale)
    % OUTPUT
    %   Imask - BW image with objects from I
    %
    % For more details see Notebook Matlab_DNA_masking1_1-22-
20  99.doc
    % Uses SDC Morphology Toolbox V0.7

    if (nargin ~= 1)
        error('Wrong number of input parameters');
25  end
    if (nargout ~= 1)
        error('Wrong number of output parameters: one output
        argument should be provided');
    end
30

    Imask = edge(I, 'canny');
    Imask = mmdil(Imask, mmsecross(1));
    Imask = mmero ( mmclohole(Imask,mmsecross(1)));

```

```

Imask = mmedgeoff(Imask, mmsecross(1));
% note that mmedgeoff this command removed FILLED OBJECTS
but not touching OUTLINES.
% these outlines can be removed by filtering:
5  Imask = medfilt2(Imask, [5 5]);

%=====end MaskDNA1 =====

```

10 Given the list of image files or montages of images as an input, this program creates an individual file for each image that contains the following quantitative measurements for all objects identified in the image:

1 - Index ("number" of an object);	8 - Solidity;
2 - X coordinate of the center of mass;	9 - Extent;
15 3 - Y coordinate "-";	10 - Total Intensity;
4 - Total Area (in pixels);	11 - Avg. Intensity;
5 - Ratio of MajorAxis/MinorAxis;	12 - Median Intensity;
6 - Eccentricity;	13 - Intensity of 20% bright pixel
7 - EquivDiameter;	14 - Intensity of 80% bright pixel

20 A fragment of an output for a single file, containing 9 images of cells stained for DNA and acquired with a 10x objective. A montage image that was used as a source to generate data in A is presented in Fig. 17.

25 The same program also summarizes measurements across many files and performs statistical analysis of the summary data. It creates a summary file with the following data:

1 - Image file number;	
2 - Average object Area (in pixels);	3 - STD (standard deviation) of
2;	
30 4 - Avg. of Ratio of MajorAxis/MinorAxis;	5 - STD of 4;
6 - Avg. Eccentricity;	7 - STD of 6;
8 - Avg. EquivDiameter;	9 - STD of 8;
10 - Avg. of Solidity;	11 - STD of 10;

- | | |
|---|----------------|
| 12 - Avg. of Extent; | 13 - STD of 11 |
| 14 - Avg. of objects Total Intensity; | 15 - STD of 14 |
| 16 - Avg. of objects Avg Intensity; | 16 - STD of 15 |
| 18 - Avg. of objects Median intensity; | 19 - STD of 18 |
| 20 - Avg. of objects intensity of 20% bright pixel; | 21 - STD of 19 |
| 22 - Avg. of objects intensity of 80% bright pixel; | 23 - STD of 21 |

An example of summary output obtained by running AnalyseDNA against 10 montage files also is shown in Appendix B.

10 A seventh experiment was conducted in order to use sequence analysis algorithms to analyze features of cell images. In this experiment, HeLa cells were treated for 24 hours with several different compounds, and then fixed, and stained with a fluorescent DNA dye. One image of these cells was acquired for each of the treatments and morphometric parameters and features were measured:

15 Resulting measurements were arranged into a string of numbers and reduced to a pseudo- nucleic acid sequence using following rules: At any given position in the sequence a number was substituted by "t" (a code for thymidine) if its value is among highest 25% of the values at the corresponding position in the data set, "g" if it is between 50% and 25%, "c" if it is between 75% and 50%, and "a" if it
20 belongs to lowest 25% of values. Thus one descriptor or sequence was generated per treatment as illustrated in Fig. 18.

Resulting sequences were clustered using an AlignX module commercial software package Vector NTI (<http://informaxinc.com>), which uses a Neighbor Joining algorithm for sequence clustering.

25 The resulting dendrogram is presented in Fig 18. On the dendrogram the closest "leafs" correspond to the closest pseudo-sequences. Interestingly, compounds with similar mechanisms of action cluster together on the dendrogram. Another example of the generation of pseudo-sequences and clustering is shown in Fig. 19.

30 In some embodiments, techniques according to the present invention can provide tools for the later stages of drug development such as clinical trial design and patient management. The properties of known drugs such as clinical trial and patient response information will be used in a similar fashion as the pre-clinical

information to provide predictions about the properties of novel compounds. Because the human cell is the locus of drug action, a database containing drug-cell interactions can be able to provide predictive information for this aspect of drug development.

Although the above has generally described the present invention
5 according to specific systems, the present invention has a much broader range of applicability. In particular, the present invention is not limited to a particular kind of data about a cell, but can be applied to virtually any cellular data where an understanding about the workings of the cell is desired. Thus, in some embodiments, the techniques of the present invention could provide information about many
10 different types or groups of cells, substances, and genetic processes of all kinds. Of course, one of ordinary skill in the art would recognize other variations, modifications, and alternatives.

APPENDIX A

EV Table 1.doc

Example of the output of AnalyseDNA.m program
(measurements for a single 3 by 3 montage image)

File#	Subimage	object#	X coord	Y coord	Area	Area ratio	Eccentricity	Equidistant	Solidity	Extant	Intensity	Avg. Intensity	Median Intensity	20% pta.	80% pta.
1	1	1	12.2897	152.655	145	1.17293	0.322616	13.5075	0.923567	0.733796	4605	31.7366	31	25	37
1	2	2	16.352	416.032	125	1.60594	0.762471	12.4157	0.905297	0.78125	4606	36.810	31	20	45
1	3	3	20.1073	73.8019	177	1.09915	0.413785	15.0121	0.917098	0.691406	4719	28.9125	29	22	31
1	4	4	21.4106	402.716	13	1.38215	0.479004	1.39229	0.914034	0.711537	3490	85.814	87	67	105
1	5	5	27.0928	184	96	1.30887	0.443194	11.0558	0.888889	0.471229	4502	16.8358	43	38	56
1	6	6	30.3252	259.534	206	1.32106	0.703209	16.1953	0.927928	0.715278	6360	30.9709	33	21	37
1	7	7	32.6429	167.573	89	1.31984	0.471654	10.4431	0.927083	0.711457	4235	12.4719	50	39	56
1	8	8	33.0411	16.9726	146	1.25176	0.401055	12.4343	0.929326	0.710718	5415	27.089	40	29	44
1	9	9	37.746	366.021	67	1.84062	0.895912	7.3578	0.87037	0.632718	667	141.053	142	113	171
1	10	10	69.1078	170.004	232	1.90491	0.451127	17.197	0.932911	0.70303	8832	42.3793	45	31	51
1	11	11	56.0749	126.534	221	1.93704	0.955555	16.7746	0.924606	0.682235	7040	31.8152	33	25	37
1	12	12	52.7755	64.9932	147	1.32627	0.463201	13.4809	0.907407	0.706721	4745	32.415	34	26	39
1	13	13	52.4444	244.854	171	1.27225	0.493953	16.7555	0.872448	0.706412	9378	54.8121	56	43	68
1	14	14	56.4029	287.272	208	1.92782	0.854916	16.1953	0.923167	0.64375	7137	31.6156	37	29	41
1	15	15	57.0616	227.176	108	1.71805	0.418039	11.2665	0.915254	0.701299	4614	43	45	31	
1	16	16	68.1714	333.181	315	1.11134	0.432246	20.0267	0.95	0.526758	13151	48.0984	50	36	62
1	17	17	65.1109	402.414	270	1.70117	0.80906	16.7366	0.920502	0.67059	9809	48.584	46	35	56
1	18	18	71.8619	443.13	163	1.72478	0.424503	15.3176	0.91133	0.64323	6174	32.1027	35	25	39
1	19	19	72.824	184.854	223	1.71588	0.812622	12.3143	0.91111	0.723529	4811	37.3577	41	30	47
1	20	20	77.6869	127.513	306	1.3379	0.791998	19.7386	0.822581	0.622231	14559	47.5781	51	38	57
1	21	21	78.7377	208.17	172	1.3357	0.462941	12.4636	0.91048	0.739394	4472	36.7159	40	29	42
1	22	22	81.4786	53.5812	117	1.67133	0.794616	12.7053	0.866166	0.602837	666	40.0313	43	32	47
1	23	23	80.7292	281.534	373	1.73386	0.887916	21.7926	0.813981	0.531339	14109	43.1877	46	34	52
1	24	24	84.1763	341.374	85	1.20799	0.540991	10.4031	0.874289	0.708233	4589	37.9882	57	43	63
1	25	25	86.1608	176.231	163	1.43533	0.717545	13.4935	0.910789	0.79444	4878	38.1119	35	27	41
1	26	26	91.4529	376.324	170	1.26853	0.463699	14.7123	0.833333	0.93233	4932	29.0176	30	23	35
1	27	27	97.7606	217.195	288	1.93233	0.862119	18.1492	0.9	0.406316	10663	37.0543	39	29	45
1	28	28	96.5841	230.363	113	1.09325	0.613609	11.9548	0.898825	0.668638	4500	40.177	43	32	48
1	29	29	96.9492	248.402	118	1.2774	0.622219	12.2323	0.921875	0.768234	4873	41.2966	43	32	51
1	30	30	103.033	93.2179	222	1.48415	0.739927	12.4636	0.915336	0.813333	4663	38.2213	40	31	47
1	31	31	103.47	155.307	124	1.3208	0.753611	13.0619	0.917808	0.691178	4358	32.3224	34	27	38
1	32	32	105.336	57.1271	110	1.90329	0.450846	12.2573	0.907492	0.691118	4593	29.7881	42	30	48
1	33	33	121.23	285.08	326	1.70219	0.809314	20.3734	0.900532	0.626923	15664	48.4626	50	39	60
1	34	34	125.532	176.645	141	1.52015	0.757019	13.3988	0.921632	0.732715	4429	45.3957	49	37	53
1	35	35	126.98	60.3355	152	1.75689	0.822706	13.9116	0.921212	0.767677	4875	45.2303	47	36	54
1	36	36	127.003	178.083	266	1.415	0.810275	18.1033	0.794607	0.528462	9810	26.8797	38	28	43
1	37	37	130.902	411.5	164	1.19276	0.51507	16.4503	0.937113	0.788462	7377	41.7378	47	35	53
1	38	38	132.439	352.545	187	1.29705	0.690312	15.4206	0.835	0.799145	5277	27.5519	27	22	31
1	39	39	129.613	16.6134	13	1.15921	0.50379	0.08843	0.920571	0.8125	56	7.20777	8	7	11
1	40	40	136.574	209.059	101	1.17013	0.450346	11.2401	0.87069	0.617436	7293	22.2029	74	58	88
1	41	41	136.455	23.0909	33	1.21119	0.564506	6.48201	0.916667	0.715714	1369	59.0606	60	41	75
1	42	42	140.873	102.008	121	1.47767	0.734306	12.4122	0.916667	0.732333	4804	29.7255	40	32	47
1	43	43	146.964	59.8199	272	1.35519	0.821825	16.6097	0.920708	0.715333	10814	39.7574	41	32	46
1	44	44	147.093	428.135	161	1.3071	0.64397	14.7175	0.923287	0.715356	9000	55.9006	56	41	68
1	45	45	151.46	256.924	274	1.08008	0.377872	16.888	0.937218	0.717778	9454	43.0182	45	33	52
1	46	46	155.688	178.546	141	1.39135	0.65293	13.3988	0.921369	0.802136	7729	54.0156	57	41	65
1	47	47	140.875	362.356	68	1.71533	0.812532	7.81744	0.853113	0.592593	6809	37.688	339	103	169
1	48	48	166.028	11.7677	194	1.42124	0.710595	15.8777	0.933942	0.732333	9331	50.1566	50	39	62
1	49	49	169.613	126.767	217	1.74118	0.820008	16.6211	0.825035	0.5425	7390	36.7742	38	28	45
1	50	50	176.014	356.114	222	1.44188	0.720419	16.8125	0.790016	0.421149	9426	43.3601	46	32	51
1	51	51	175.748	192.983	110	1.13214	0.47708	12.2573	0.900743	0.698125	4671	39.5842	42	32	47
1	52	52	177.181	210.678	127	1.16539	0.467606	12.7122	0.92029	0.755952	4862	38.2835	40	30	45
1	53	53	178.767	410.524	167	1.24127	0.592425	13.4609	0.920378	0.75	9094	61.9776	67	50	72
1	54	54	182.4	372.176	170	1.37768	0.755987	16.7133	0.932277	0.716486	9235	54.3118	58	44	66
1	55	55	189.186	262.719	196	1.01704	0.816976	15.7973	0.915408	0.712424	5030	25.6623	27	20	31
1	56	56	200.742	93.7118	213	1.36923	0.683428	16.4682	0.912118	0.717368	9465	65.3756	66	38	53
1	57	57	199.198	156.723	91	1.01652	0.295161	10.7611	0.90059	0.752066	4188	16.022	47	36	56
1	58	58	209.47	183.871	244	1.91053	0.640182	18.334	0.916667	0.5	9873	37.3977	39	32	46
1	59	59	208.757	70.0135	230	1.43139	0.806478	17.1127	0.916502	0.804196	9166	42.609	44	31	53
1	60	60	212.384	348.659	197	1.12679	0.723587	15.8376	0.928245	0.724763	7014	35.6041	26	21	44
1	61	61	220.956	20.6237	194	1.27662	0.923588	15.7165	0.92823	0.774	4335	23.9118	28	21	29
1	62	62	216.588	236.39	183	1.31183	0.666783	15.2644	0.912299	0.826054	5078	27.7186	28	21	34
1	63	63	218.292	293.953	171	1.41637	0.79437	14.7455	0.919355	0.492308	5053	25.5197	30	23	35
1	64	64	217.331	330.721	172	1.72537	0.41481	16.7986	0.924731	0.471875	5010	28.2023	31	22	36
1	65	65	217.306	421.1	201	1.79372	0.634418	18.9915	0.924267	0.728971	4084	30.3184	32	25	36
1	66	66	222.024	157.769	121	1.7261	0.656753	12.4122	0.916467	0.732333	4352	35.9169	38	29	42
1	67	67	249.071	486.968	135	1.20117	0.549955	23.5312	0.74358	0.402778	14810	36.016	37	26	42
1	68	68	238.814												

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49	243.509	88.7857	224	1.47991	0.846762	16.808	0.899598	0.836364	8827	39.4063	42	49
50	246.931	322.144	160	1.79881	0.820819	14.273	0.91954	0.76555	3025	31.1063	33	50
51	249.238	413.026	77	1.51864	0.491551	9.90149	0.875	0.7	4352	56.3193	39	51
52	256.963	43.816	163	1.58937	0.777002	14.4062	0.91373	0.479167	4720	28.9371	30	52
53	257.061	398.848	66	1.03119	0.245208	9.167	0.916617	0.414813	4110	67.2727	71	53
54	263.602	375.59	251	1.95991	0.860039	13.8769	0.80826	0.597619	10500	61.0727	63	54
55	264.231	213.001	161	1.45084	0.295632	14.3175	0.914773	0.488031	5126	31.9006	33	55
56	266.937	209.807	131	1.21512	0.649432	11.8802	0.917355	0.720779	4858	43.7658	45	56
57	276.137	348.278	131	1.58183	0.771829	13.9119	0.90722	0.482291	9633	33.3144	77	57
58	276.221	171.26	204	1.05413	0.873763	16.1165	0.918919	0.596491	7051	36.5337	35	58
59	277.039	285.098	287	1.77833	0.823935	13.118	0.87231	0.450191	10320	36.4531	38	59
60	277.327	97.32	150	1.10221	0.422329	13.8196	0.92045	0.765206	9202	81.3167	63	60
61	276.612	391.118	85	1.67318	0.801747	10.4031	0.922313	0.817301	4387	51.4118	55	61
62	285.905	151.719	221	1.56301	0.74855	17.1419	0.931432	0.675439	6580	37.1629	39	62
63	285.326	203.688	221	1.75639	0.822032	16.7716	0.963281	0.701581	10251	46.3016	49	63
64	284.739	355.022	66	1.74017	0.810396	7.45308	0.861923	0.730159	6986	34.069	35	64
65	291.4	319.71	165	1.31361	0.640467	13.5875	0.917722	0.735208	5972	31.1042	32	65
66	293.631	442.726	152	2.0131	0.640208	15.6353	0.917722	0.735208	5972	31.1042	32	66
67	293.81	383.276	58	1.35716	0.60351	8.59318	0.920615	0.735	3946	48.3488	70	67
68	299.102	285.182	159	1.39289	0.696112	11.2243	0.929226	0.737143	5103	32.0943	33	68
69	300.14	356.247	150	1.31518	0.649643	13.8138	0.925926	0.78125	5319	35.7933	37	69
70	311.3	280.38	382	1.53517	0.771632	22.054	0.91267	0.598746	14117	42.1911	44	70
71	308.77	132.891	161	1.13063	0.69165	11.3175	0.935017	0.746647	4965	30.8417	33	71
72	317.161	23.8111	160	1.47884	0.789104	13.1318	0.932642	0.728743	4915	27.2036	24	72
73	309.682	206.548	126	1.8418	0.733615	12.664	0.9	0.7	1950	39.2833	40	73
74	317.32	43.06	150	1.19043	0.64863	13.8138	0.982333	0.595228	1958	37.0333	35	74
75	315.779	227.448	165	1.2165	0.569542	13.5875	0.917722	0.735208	5972	31.1042	32	75
76	314.612	396.048	167	1.76345	0.820055	12.6809	0.936306	0.772681	9195	41.551	63	76
77	319.371	79.6031	213	1.37819	0.648296	17.5897	0.915325	0.759335	8192	33.7119	36	77
78	321.595	171.65	207	1.17294	0.52283	16.2343	0.928231	0.741028	4933	24.8208	33	78
79	321.232	313.626	89	1.37851	0.623916	11.3232	0.937862	0.781538	6632	48.7879	50	79
80	328.695	665.233	153	1.81993	0.788719	13.9533	0.9	0.7	1950	39.2833	40	80
81	339.875	30.4074	328	2.08737	0.877601	20.4338	0.996175	0.591203	10208	31.122	33	81
82	338.286	341.101	168	1.34659	0.681377	14.6255	0.928177	0.716667	4848	28.8371	29	82
83	335.277	128.088	177	1.47067	0.732241	13.2073	0.901316	0.713548	4912	50.5985	33	83
84	336.608	376.592	130	1.44642	0.722401	12.8655	0.920778	0.666687	1713	36.2338	38	84
85	340.273	422.859	128	1.81993	0.837097	12.7642	0.920843	0.64811	1609	34.0078	38	85
86	341.621	397.101	149	1.31333	0.548431	13.7336	0.923464	0.746103	1920	33.0201	33	86
87	352.93	231.248	201	1.50456	0.717942	15.9975	0.922018	0.772077	8187	42.2239	43	87
88	352.582	107.863	370	1.46075	0.643317	13.9116	0.944318	0.536078	5109	33.4118	35	88
89	360.316	246.068	152	1.20418	0.557215	11.0215	0.981496	0.644441	4620	39.8776	41	89
90	357.882	416.327	110	1.31165	0.719222	12.353	0.923333	0.777778	4854	34.6714	35	90
91	361.836	316.403	116	1.27316	0.618912	13.3512	0.923333	0.777778	4854	34.6714	35	91
92	361.357	65	110	1.27316	0.618912	13.3512	0.923333	0.777778	4854	34.6714	35	92
93	363.058	287.398	103	1.26778	0.614641	11.4518	0.927228	0.792308	4724	49.8441	40	93
94	377.556	52.6892	303	2.26781	0.895101	22.0828	0.951111	0.526099	12083	31.5183	20	94
95	369.901	316.09	111	1.23541	0.656722	11.8882	0.909826	0.772224	4428	39.8919	41	95
96	372.355	389.158	183	1.08117	0.392338	15.2644	0.953125	0.871029	1873	25.5155	28	96
97	373.696	102.31	188	1.77426	0.72647	14.8255	0.913043	0.658824	6590	39.2342	41	97
98	377.238	164.559	172	1.55567	0.766024	16.7986	0.910053	0.67451	5008	29.1163	30	98
99	378.303	487.237	190	1.34389	0.658045	15.5536	0.913462	0.698329	6078	31.9855	32	99
100	386.803	129.803	112	1.83726	0.836939	13.4482	0.940297	0.788889	8149	57.3473	61	100
101	387.01	222.832	206	1.51589	0.751367	16.1953	0.923767	0.646667	4561	47.5417	48	101
102	384.354	303.715	36	1.73508	0.819527	11.0558	0.923077	0.646667	4561	47.5417	48	102
103	397.856	600.719	313	1.71338	0.812011	19.9831	0.900512	0.610136	14036	44.8435	46	103
104	399.741	281.944	108	1.86616	0.844617	11.7245	0.892562	0.593407	1668	43.2222	44	104
105	392.248	318.782	105	1.73731	0.742673	11.5674	0.913043	0.673077	4633	43.9333	46	105
106	393.708	20.375	120	1.05184	0.34362	12.3609	0.916031	0.763231	4538	37.8167	39	106
107	402.593	198.495	196	1.83729	0.838503	15.7145	0.922691	0.769841	9702	50.0103	52	107
108	402.542	362.046	190	2.18162	0.897103	12.8655	0.902778	0.601852	1566	35.1231	37	108
109	401.298	33.0781	121	1.34189	0.646895	12.4122	0.908774	0.720738	4622	38.1981	41	109
110	401.174	319.356	109	1.24316	0.610958	11.7806	0.908333	0.698718	4133	40.4697	42	110
111	418.441	432.387	142	2.10337	0.879632	13.4462	0.899734	0.71	4284	30.149	32	111
112	408.948	170.017	174	1.83507	0.717735	11.8843	0.910995	0.74359	8845	37.8418	39	112
113	415.673	278.164	147	1.34843	0.613618	13.4809	0.910318	0.765625	4576	33.6503	36	113
114	419.981	320.856	119	1.70251	0.809316	12.2373	0.887218	0.605128	4596	38.9492	41	114
115	424.797	224.645	172	1.24283	0.553793	11.7386	0.93373	0.614414	5120	39.7674	32	115
116	428.433	38.3016	126	1.24189	0.593057	12.666	0.926471	0.71	4585	36.3869	39	116
117	431.977	106.932	222	1.74332	0.818891	16.8125	0.925	0.720378	7043	31.7252	33	117
118	432.609	424.282	131	1.70121	0.808793	11.9113	0.927535	0.770368	4251	31.6504	31	118
119	431.355	471.519	179	1.44151	0.62725	12.6159	0.918846	0.747037	4387	34.0078	35	119
120	418.675	12.7222	124	1.23999	0.624707	12.648	0.922333	0.75	4465	35.2718	36	120
121	413.29	664.766	124	1.33433	0.642198	12.5451	0.9184319	0.688889	4858	37.5415	40	121
122	443.287	152.375	124	1.38301	0.690747	12.119	0.913737	0.708232	5017	36.8497	39	122
123	432.32	495.598	106	1.66314	0.7199015	19.7386	0.918919	0.756875	17326	57.9781	61	123
124	431.122	370.49	288	1.71678	0.826502	19.1492	0.823215	0.659019	11368	39.5417	42	124

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1	165	462.718	316.932	291	2.5982	0.923028	19.7487	0.716719	0.534928	9565	37.9694	32	23	43
2	166	455.84	315.437	319	1.51711	0.752012	12.3092	0.732333	473	37.5882	39	28	46	
3	167	455.357	316.335	336	1.77445	0.816825	14.0028	0.921336	0.712333	9038	37.1948	55	42	51
4	168	459.428	310.199	201	1.77445	0.739188	18.5975	0.934886	0.755439	4618	27.9602	34	16	27
5	169	462.472	313.039	327	1.2763	0.613731	22.7182	0.967761	0.874675	4819	38.1811	60	30	40
6	170	465.054	324.432	403	1.27102	0.634423	22.7082	0.98404	0.774439	5811	26.2247	23	19	29
7	171	456	323	23	1.22322	0.577798	5.41132	0.851832	0.453113	2887	116.926	118	86	135
8	172	469.289	323.606	226	1.65152	0.796338	17.0302	0.913255	0.890909	9119	39.8219	37	28	41
9	173	469.291	365.372	117	1.72286	0.683137	12.2033	0.936	0.873214	4533	29.9145	40	32	45
10	174	469.161	366.176	23	1.72286	0.523119	5.41132	0.92	0.716881	2572	111.326	115	97	136
11	175	477.09	365.403	199	1.22807	0.646434	15.9137	0.947619	0.737037	7638	38.3019	40	31	46
12	176	480.109	321.381	130	1.71131	0.773573	12.2555	0.926174	0.700032	4516	27.7101	31	27	39
13	177	485.215	312.969	163	1.99816	0.865802	14.4062	0.913723	0.679367	6781	41.6226	43	32	51
14	178	492.535	360.986	129	1.6323	0.730838	12.8159	0.921829	0.716663	1590	35.5914	37	28	44
15	179	498.198	357.492	197	1.18157	0.536017	15.8276	0.933619	0.749531	6328	35.5914	37	28	44
16	180	498.107	428.713	122	1.39074	0.638967	12.4634	0.930448	0.739394	6778	39.1639	40	31	48
17	181	501.794	480.379	107	1.30083	0.639539	11.672	0.938596	0.740058	4201	40.1963	42	32	47
18	182	48.0123	189.207	150	1.82313	0.80851	13.8198	0.9375	0.739976	5013	33.42	35	26	40
19	183	37.3182	92.819	126	1.24311	0.595788	12.646	0.919708	0.73	1636	26.7937	29	21	44
20	184	37.7436	251.949	70	1.23486	0.588585	9.96557	0.906977	0.707879	1177	53.5513	54	42	63
21	185	21.4605	27.3122	352	2.05915	0.876151	13.9116	0.915663	0.591751	3399	35.5392	38	29	42
22	186	21.4605	152.717	180	1.431069	0.705237	13.1386	0.928077	0.712286	7613	42.2914	45	33	52
23	187	30.9013	150.013	151	1.5312	0.757225	12.8658	0.937888	0.607487	6836	32.0132	34	25	39
24	188	35.2216	117.641	153	1.10365	0.43208	13.5573	0.916168	0.735331	5847	57.8235	60	46	70
25	189	32.0386	482.965	57	1.17959	0.510388	13.9108	0.901782	0.716647	7839	27.8256	12	104	175
26	190	41.1142	68.0531	254	2.15973	0.816332	17.9836	0.921907	0.735848	8532	37.5906	36	27	39
27	191	46.0943	205.164</											

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1	1	136	483.48	366.469	339	2.90072	0.934085	19.3115	0.95531	0.68891	14063	47.0101	50	30	37
1	2	137	488.732	106.247	190	1.73179	0.821058	15.3536	0.95556	0.66667	8786	46.2421	50	35	37
1	3	138	492.338	317.207	193	1.3628	0.679385	15.757	0.933014	0.77281	5508	29.7322	29	23	31
1	4	139	50.098	359.078	102	1.21521	0.56818	11.3961	0.918019	0.733287	5508	34	38	39	40
1	5	140	119.565	117.543	117	1.8992	0.850152	13.6009	0.880281	0.764771	5099	34.4871	37	25	44
1	6	141	16.317	278.214	176	1.49674	0.714054	14.9696	0.926110	0.647039	9866	56.0568	60	45	68
1	7	142	15	276.431	82	1.20278	0.562886	10.2179	0.88137	0.603233	4220	52.8024	56	45	64
1	8	143	22.1517	323.427	211	1.49712	0.714106	16.3907	0.905579	0.703333	9330	53.3028	67	35	55
1	9	144	22.7797	353.041	74	1.16551	0.513637	9.70668	0.880532	0.747475	4079	55.1216	56	44	66
1	10	145	22.7797	353.041	69	1.47558	0.723029	9.7302	0.880532	0.747475	3983	57.7216	59	48	10
1	11	146	22.7797	353.041	135	1.63061	0.788873	16.0182	0.933733	0.591861	4887	31.5329	33	24	39
1	12	147	44.2723	455.718	233	1.9178	0.864286	11.224	0.889403	0.647222	9769	60.2103	42	31	50
1	13	148	51.8162	275.266	191	1.67147	0.80208	15.5945	0.863214	0.636667	9954	52.1152	54	42	62
1	14	149	62.4292	261.297	219	1.22958	0.581065	16.4682	0.862205	0.607333	9758	44.5818	46	36	56
1	15	150	65.3559	171.246	215	2.19722	0.890325	16.4682	0.864667	0.608810	7186	35.1455	36	27	63
1	16	151	62.4132	488.702	121	1.68052	0.806179	12.4122	0.923684	0.75825	4267	35.2415	37	28	63
1	17	152	73.3548	40.742	135	1.30261	0.61153	14.0182	0.923684	0.75825	4267	59.871	61	50	71
1	18	153	79	329.532	103	1.97089	0.861719	15.2464	0.915	0.72619	4967	22.1121	28	21	33
1	19	154	80.0786	183.618	210	1.64688	0.794513	16.3518	0.928204	0.714286	7237	36.3371	37	29	41
1	20	155	79.6594	294.823	341	1.34667	0.603919	13.3988	0.932773	0.703233	4820	31.7553	36	27	40
1	21	156	80.1048	310.149	228	1.39515	0.67472	12.5551	0.925273	0.751515	4607	37.1522	38	30	44
1	22	157	86.0286	103.431	168	1.53706	0.766515	14.4503	0.918019	0.68078	5296	37.2927	34	25	32
1	23	158	90.3161	139.787	174	1.67023	0.80086	14.8843	0.920635	0.651135	4650	26.954	29	21	29
1	24	159	95.1818	304.591	27	1.58172	0.771787	13.29237	0.916887	0.732323	1517	48.9515	61	64	76
1	25	160	88.1814	72.4166	63	1.88079	0.646635	7.39928	0.914894	0.671875	6442	149.037	133	186	186
1	26	161	95.9063	437.878	188	1.45559	0.721051	15.4716	0.930893	0.705769	4230	23.0319	32	18	26
1	27	162	103.773	259.661	271	2.25576	0.896369	10.5735	0.73882	0.531233	10182	38.2362	40	30	47
1	28	163	101.716	351.303	109	1.34599	0.680632	11.7806	0.92329	0.776371	6566	41.4893	44	33	32
1	29	164	100.771	381.4	105	1.68452	0.804731	11.5074	0.912012	0.678079	4538	42.219	45	34	33
1	30	165	102.731	26.3077	182	1.45701	0.729319	12.2271	0.938166	0.722222	5225	29.3132	31	24	35
1	31	166	108.742	241.331	232	1.52887	0.756126	17.9125	0.818182	0.666667	10076	39.9041	43	31	49
1	32	167	107.882	297.916	120	1.2305	0.58271	12.1955	0.928571	0.727222	4831	37.1615	37	29	46
1	33	168	112.68	139.313	128	1.2113	0.564315	12.7662	0.901578	0.761905	4503	35.1797	38	28	42
1	34	169	112.676	420.502	145	1.52776	0.759681	13.5075	0.91195	0.710788	3962	27.331	28	21	34
1	35	170	119.59	274.808	104	1.58258	0.775068	11.5073	0.912281	0.675225	4622	41.4123	43	34	35
1	36	171	124.621	445.212	160	1.13828	0.683005	14.273	0.85615	0.686647	3953	24.7063	35	19	31
1	37	172	123.561	492.612	133	1.16798	0.691128	13.0131	0.93001	0.730769	3821	28.7293	30	23	31
1	38	173	129.158	286.61	114	1.81038	0.833599	12.0178	0.919353	0.781687	4570	40.0877	42	31	43
1	39	174	134.635	412.167	80	1.7702	0.823154	10.9225	0.898276	0.714286	4315	52.9375	53	42	47
1	40	175	144.778	93.2454	216	1.26728	0.614272	15.5937	0.925065	0.75	8028	37.1687	38	20	44
1	41	176	141.008	261.556	314	1.78966	0.829377	12.0078	0.912	0.626371	4590	40.2632	42	32	49
1	42	177	147.37	232.151	119	1.2221	0.574039	12.1092	0.922481	0.727272	4226	69.1261	72	52	62
1	43	178	150.157	435.038	102	1.36069	0.678155	11.3961	0.910714	0.712287	3854	37.7883	40	31	45
1	44	179	154.635	412.167	108	1.47096	0.733773	11.7265	0.915254	0.701299	4156	38.4951	40	30	46
1	45	180	158.645	295.772	254	2.29558	0.90072	17.9834	0.84206	0.529167	9778	38.4951	40	31	48
1	46	181	159.39	30.2537	201	1.64383	0.793681	15.9775	0.916884	0.705263	4592	36.7161	36	27	42
1	47	182	159.759	272.021	144	1.23912	0.590519	13.5406	0.923077	0.738162	9195	62.8512	67	52	78
1	48	183	166.537	390.408	287	1.65549	0.798846	18.4379	0.946809	0.796643	10321	39.4065	41	29	49
1	49	184	170.118	427.126	119	2.42927	0.771157	12.2092	0.915285	0.703233	4156	36.9244	36	26	43
1	50	185	174.639	421.702	181	1.36362	0.679866	15.5945	0.915545	0.757937	4288	43.9162	45	36	52
1	51	186	175.666	172.404	154	1.35535	0.673001	11.0028	0.927711	0.733233	4793	44.1104	46	34	51
1	52	187	182.212	71.0718	153	2.41914	0.709551	12.9573	0.921687	0.75	4953	32.3725	34	26	38
1	53	188	188.446	428.314	144	2.16718	0.884928	13.5406	0.93411	0.571129	4506	31.2917	33	23	39
1	54	189	196.816	172.408	293	1.59008	0.777488	19.3117	0.904121	0.636937	21219	72.5222	74	53	89
1	55	190	191.89	340.358	286	1.78224	0.827754	18.0826	0.925566	0.732323	10774	35.9701	37	29	43
1	56	191	202.502	375.631	293	2.40846	0.903729	19.3117	0.91438	0.631466	10074	34.2115	36	26	42
1	57	192	206.056	40.8423	222	1.22324	0.654892	16.4125	0.926709	0.719347	9681	43.6081	46	34	53
1	58	193	218.937	319.988	269	1.78272	0.827854	18.5060	0.996667	0.68798	10198	37.9109	40	30	45
1	59	194	217.344	409.156	250	1.92724	0.854357	17.8112	0.916993	0.548246	3609	38.436	39	28	48
1	60	195	215.452	102.911	157	1.45643	0.727023	11.1386	0.928981	0.769608	6078	38.7134	40	31	46
1	61	196	221.224	168.337	304	1.81246	0.834019	18.474	0.87106	0.603175	70052	65.9605	70	51	81
1	62	197	224.312	36.0872	149	1.27631	0.621533	13.7126	0.92175	0.776042	4599	30.8658	32	25	36
1	63	198	228.916	451.459	159	1.30781	0.693392	14.2293	0.924619	0.719457	4908	30.8679	32	25	37
1	64	199	236.586	365.855	237	1.34178	0.668762	17.2712	0.946429	0.653233	5945	24.6824	35	18	31
1	65	200	235.133	13.5773	97	1.53129	0.758418	11.1132	0.941748	0.768041	4282	44.1163	47	32	34
1	66	201	238.177	51.8398	181	1.30202	0.641115	15.1808	0.92805	0.760501	4719	26.2316	28	21	32
1	67	202	242.074	491.718	189	1.66383	0.799233	15.5126	0.935444	0.726921	4786	25.3728	26	21	30
1	68	203	238.109	254.516	128	1.27024	0.61671	12.7642	0.914166	0.761905	5025	39.2578	41	31	43
1	69	204	245.439	301.019	283	1.71339	0.87214	19.0192	0.910394	0.652176	8887	31.1173	32	23	37
1	70	205	244.546	445.491	155	1.6373	0.68906	14.0182	0.922419	0.717593	4694	30.2039	32	25	36
1	71	206	252.381	36.7031	110	1.03333	0.334139	11.8315	0.92437	0.761889	4358	39.6273	41	33	48
1	72	207	260.993	89.3091	139	1.83152	0.854033	13.2034	0.902997	0.571167	4647	32.4317	34	26	41
1	73	208	240.8	238.807	79	1.34181	0.691768	9.77203	0.882353	0.691446	8194	109.213	108	86	132
1	74	209	260.881	165.982	59	1.48172	0.804	6.6724	0.867667	0.58596	7107	170.458	121	74	167
1	75	210	265.891	16.1091	110	1.34948	0.671725	11.6345	0.89427	0.769231	1628	42.0727	44	33	51

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1	150	693.585	286.084	35	1.18928	0.341285	6.47558	0.945916	0.833232	3545	101.286	105	89	117
1	151	697.438	76.3769	320	2.04206	0.571891	12.7655	0.902778	0.637255	4533	35.0231	36	26	47
1	152	699.826	146.508	328	1.24075	0.321863	12.7662	0.920863	0.711111	4858	37.9375	39	30	46
1	153	506.436	285.482	39	1.4304	0.715017	7.04673	0.906977	0.8125	3400	94.359	96	79	113
1	1	20	122.671	152	1.3561	0.675419	13.0116	0.929245	0.730789	4832	31.7855	33	75	39
1	2	22.2091	72.3198	197	1.32473	0.643237	13.8376	0.929245	0.730789	4832	48.1726	50	37	59
1	3	22.1391	148.043	315	1.35777	0.576423	12.1005	0.92	0.716733	4511	39.2761	40	20	48
1	4	23.2776	287.022	137	1.66819	0.600412	13.7072	0.909619	0.805892	6042	44.1022	47	35	53
1	5	26.6026	348.243	151	1.68495	0.604842	13.6658	0.909619	0.805892	6042	44.1022	47	35	53
1	6	28.0294	163.558	102	1.20299	0.626193	11.3961	0.93378	0.746415	4522	44.4316	45	36	53
1	7	26.731	189.474	282	2.20222	0.698891	18.9487	0.817391	0.321637	9639	31.3182	35	26	43
1	8	39.3951	189.77	103	2.26982	0.906608	15.7441	0.905911	0.518935	4754	24.0929	27	19	33
1	9	45.5199	217.316	377	2.90367	0.939016	21.3092	0.821131	0.419235	15115	40.8886	35	26	60
1	10	60.3109	306.044	119	1.46635	0.731385	12.3092	0.929688	0.732333	4745	39.8739	41	29	48
1	11	69.8224	424.27	239	2.13833	0.803911	16.1595	0.799383	0.535174	10090	38.9375	41	30	48
1	12	57.6559	76.3118	186	1.22662	0.579108	13.789	0.944162	0.701513	9459	50.9084	53	42	60
1	13	61.75	496.487	154	1.3807	0.689518	14.0935	0.928571	0.764706	6401	41.0321	42	29	51
1	14	61.8438	110.813	96	1.5445	0.768035	11.0358	0.997196	0.671329	4500	44.875	49	38	56
1	15	70.4453	190.465	172	2.30563	0.901017	14.7986	0.900324	0.532508	5838	35.9419	35	27	41
1	16	69.6916	97.7778	81	1.43609	0.717712	10.3554	0.9	0.75	4431	31.7037	58	41	85
1	17	87.8529	100.161	102	1.86101	0.813361	11.7961	0.894737	0.708323	4368	41.7843	46	33	57
1	18	89.9259	68.8168	108	1.19331	0.491595	11.7265	0.915254	0.755245	4387	40.4204	43	33	46
1	19	98.8812	183.476	190	2.31215	0.901662	15.5536	0.92232	0.688108	7738	48.7158	42	32	49
1	20	98.6416	473.739	251	1.39113	0.695175	17.8749	0.833016	0.747024	10187	42.9761	46	34	51
1	21	96.8018	353.413	111	1.48667	0.805287	11.8882	0.917355	0.71	668	42.2167	44	31	51
1	22	96.6612	405.321	121	1.21328	0.640287	12.4122	0.916667	0.705774	4595	37.9752	41	29	46
1	23	98.7228	41.5248	103	1.32405	0.716833	11.3401	0.90981	0.765152	4325	42.8218	44	33	52
1	24	100.188	110.136	176	1.48194	0.738011	11.9596	0.928316	0.739498	5059	28.7163	29	22	36
1	25	101.745	300.07	115	1.46323	0.817716	12.1005	0.948163	0.589144	4500	38.6522	41	31	49
1	26	108.133	181.793	135	1.85071	0.933452	13.1106	0.918167	0.711758	4866	36.0414	36	28	45
1	27	119.875	413.708	68	2.28411	0.899068	7.81766	0.774194	0.672377	5782	39.1339	41	31	47
1	28	124.703	39.2879	112	1.53298	0.85096	11.9416	0.88189	0.622222	4783	40.9	43	33	49
1	29	123.518	239.373	110	1.58153	0.763275	11.8318	0.92377	0.705128	488	40.9	43	33	49
1	30	121.016	410.145	128	1.20718	0.670133	12.7162	0.907801	0.741205	4536	37.082	28	29	45
1	31	130.984	90.248	125	1.35859	0.678922	12.6157	0.925326	0.757578	4536	37.082	28	29	45
1	32	135.816	109.582	182	1.19473	0.551636	15.2227	0.925371	0.748708	5123	28.1484	29	22	36
1	33	140.518	124.195	164	1.48308	0.803354	14.4502	0.921318	0.739258	4956	30.2195	31	24	36
1	34	146.5	164.779	101	1.35353	0.603398	11.5073	0.913281	0.732322	4704	42.3462	44	35	50
1	35	151.316	285.803	178	1.35514	0.678036	12.7682	0.907801	0.711111	4598	36.7031	39	30	44
1	36	154.718	473.329	157	1.45981	0.788134	14.1316	0.928894	0.654167	488	30.879	31	25	38
1	37	153.258	431.715	116	1.73133	0.816376	12.153	0.892308	0.644164	4545	39.181	38	29	49
1	38	157.479	252.556	110	1.53518	0.756933	13.3512	0.915033	0.729167	4828	36.4857	35	27	43
1	39	160.654	163.442	156	1.03336	0.232051	14.0935	0.923077	0.742837	9409	61.5962	64	49	73
1	40	165.455	161	121	1.71973	0.813136	12.4122	0.898296	0.664035	4553	37.4281	38	29	46
1	41	164.835	45.5079	63	1.72872	0.815228	8.95623	0.913043	0.63	7016	111.265	116	91	136
1	42	168.561	331.11	92	1.63974	0.735517	10.7178	0.87274	0.671212	4075	19.5951	52	40	60
1	43	167.286	389.735	98	1.61962	0.786637	11.7104	0.883883	0.7	4307	42.349	46	35	52
1	44	171.568	92.6129	261	2.5791	0.921732	20.8169	0.837741	0.445823	14949	43.087	45	33	55
1	45	170.147	210.678	87	1.71387	0.558844	10.5248	0.915789	0.790909	4778	54.3155	56	43	65
1	46	172.538	433.189	130	1.6118	0.72038	12.8655	0.915493	0.711286	4816	37.0162	37	29	46
1	47	182.658	195.457	81	1.03332	0.238957	10.1534	0.94186	0.81	4816	39.058	40	45	71
1	48	188.789	451.267	194	1.86937	0.818831	15.7165	0.941716	0.734848	5054	26.0515	27	20	32
1	49	197.423	222.729	579	1.51599	0.732903	27.1515	0.772	0.486555	24028	41.4991	42	31	53
1	50	201.182	275.591	154	1.34856	0.678916	14.0038	0.933323	0.802082	7416	48.1558	51	31	56
1	51	208.366	39.4718	112	1.39993	0.698817	13.4482	0.927078	0.739383	4162	31.4225	32	26	39
1	52	216.594	153.421	254	1.67351	0.801835	17.9834	0.895327	0.634591	47587	49.5551	51	37	62
1	53	216.18	292.583	123	1.23562	0.587384	12.5113	0.911111	0.722183	7499	60.9675	63	51	73
1	54	223.36	332.161	125	1.10482	0.435139	12.6157	0.93985	0.801282	4474	37.392	38	29	44
1	55	224.5	17	8	1.19372	0.517232	3.19154	0.8	0.666667	82	10.75	11	6	14
1	56	235.055	181.581	261	2.2866	0.895301	17.5172	0.838806	0.617948	7444	30.888	32	22	39
1	57	230.172	102.352	170	1.70362	0.536529	12.7682	0.907801	0.63461	5212	40.3188	41	31	50
1	58	236.018	79.3822	167	1.60813	0.701132	14.5819	0.922632	0.755156	4581	27.1311	29	22	37
1	59	238.949	205.641	117	1.271	0.617231	12.2053	0.978571	0.75971	4816	39.453	41	30	48
1	60	241.222	378.852	81	1.37305	0.684378	10.1534	0.89011	0.75	6018	74.5122	78	59	82
1	61	252.197	247.41	265	3.61941	0.961025	22.1404	0.83149	0.49359	11357	28.0887	31	23	36
1	62	259.684	354.2	95	1.16027	0.507188	10.5981	0.88785	0.719497	4255	43.2103	47	38	54
1	63	288.422	143.038	215	1.63188	0.87959	20.0267	0.791457	0.486275	9714	30.9281	33	34	37
1	64	272.363	234.679	256	1.37039	0.711011	18.0541	0.911032	0.711111	8749	34.1758	34	28	43
1	65	275.955	243.084	92	1.42816	0.71438	10.6817	0.894231	0.704315	4271	46.4916	47	36	57
1	66	275.221	372.134	199	1.5215	0.734659	19.9177	0.925561	0.69072	4521	22.7186	21	18	27
1	67	271.004	372.134	97	1.98715	0.841168	11.1132	0.873874	0.373564	4365	45	46	36	55
1	68	278.578	102.531	109	1.66495	0.720779	11.7806	0.886178	0.698716	5405	49.5472	50	37	63
1	69	278.534	205.402	103	1.37098	0.481011	11.4518	0.883931	0.72028	4111	41.0252	44	32	52
1	70	288.613	488.523	261	2.99716	0.942731	16.2295	0.853941	0.466071	9397	35.6707	36	27	46
1	71	283.109	451.764	56	1.6825	0.8085	8.76828	0.872016	0.411111	6359	115.618	120	91	142
1	72	290.576	78.3773	165	1.96088	0.840189	14.4913	0.932203	0.75	5053	30.6242	32	24	34

EV Table 1.doc

1	1	100.012	29.1471	170	1.2847	0.52779	14.3122	0.303031	0.674603	4399	23.0471	21	22
2	1	106.881	874.326	126	1.37011	0.851846	17.466	0.872617	0.5	4180	35.5536	28	43
3	1	310.799	253.649	299	1.47883	0.744490	19.3115	0.811313	0.719515	20353	68.0702	33	81
4	1	323.322	169.374	266	1.56218	0.768264	19.4774	0.817314	0.62037	9221	36.6453	39	27
5	1	320.089	159.319	169	1.58065	0.774437	19.8889	0.818471	0.722232	7357	43.7697	41	33
6	1	321.261	132.151	119	1.62076	0.789319	22.2092	0.838608	0.701142	6178	39.1109	41	38
7	1	331.813	612.721	123	1.51786	0.7523	17.3163	0.838931	0.82	1675	37.6016	31	44
8	1	301.061	66.914	356	1.81033	0.845266	21.3639	0.813628	0.367819	15309	42.7428	33	33
9	1	310.704	254.099	233	2.05326	0.874056	17.274	0.814603	0.715923	6844	38.1132	31	35
10	1	346.781	107.539	236	2.93122	0.960843	19.0341	0.816845	0.613169	7926	30.9409	33	36
11	1	346.119	140.437	160	1.20679	0.593737	11.271	0.811284	0.714226	3574	37.0015	39	35
12	1	350.881	77.437	151	1.13383	0.471236	13.8438	0.808639	0.710048	9103	35.1912	34	41
13	1	331.813	672.913	138	1.51645	0.772395	13.2355	0.82	0.737166	6117	34.1812	35	41
14	1	370.359	149.906	137	1.21531	0.420262	12.2053	0.811053	0.73574	6116	39.131	41	31
15	1	374.414	160.011	225	1.41901	0.710104	16.3351	0.823337	0.765306	8824	41.6432	45	31
16	1	374.337	304.93	86	1.23773	0.607318	10.4482	0.803763	0.781188	3641	44.6478	48	34
17	1	389.655	89.1184	190	1.2473	0.473721	15.3316	0.817467	0.765091	6740	35.1737	31	28
18	1	397.75	265.233	186	1.51957	0.850216	15.3389	0.810372	0.614281	8465	35.3104	41	35
19	1	381.991	323.973	111	1.61904	0.74446	11.8882	0.825	0.812222	8218	34	35	30
20	1	409.164	203.281	229	1.49096	0.804392	11.0353	0.816291	0.600699	9687	42.3013	41	31
21	1	406.944	231.4231	77	1.36856	0.801537	9.50169	0.830617	0.835	7522	37.6833	37	78
22	1	419.233	248.542	236	1.74772	0.820132	21.2302	0.825319	0.585526	32819	36.0084	37	88
23	1	408.808	14.7345	13	1.55343	0.435165	9.4088	0.80214	0.737374	7784	106.63	111	123
24	1	416.795	387.345	161	2.4239	0.911038	14.3175	0.844446	0.326164	5442	34.2837	37	46
25	1	414.418	81.1193	107	1.73167	0.535813	9.72118	0.805403	0.781666	4024	60.0597	42	47
26	1	412.019	191	107	2.44142	0.936943	11.472	0.829457	0.64875	3463	36.1309	34	23
27	1	420.848	128.549	161	1.6173	0.708939	13.5106	0.817197	0.75	4450	21.1806	33	21
28	1	421.104	104.037	106	1.43149	0.316964	11.6174	0.808303	0.627219	4077	38.4623	35	21
29	1	426.239	160.553	188	1.43937	0.319232	15.4714	0.830693	0.734375	8843	17.0332	49	36
30	1	425.826	321.058	169	2.88936	0.934199	18.4042	0.835837	0.479187	7277	50.4774	48	35
31	1	438.319	109.233	116	1.445	0.72186	12.153	0.813316	0.70303	4171	35.9359	38	27
32	1	443.092	158.708	120	1.88149	0.73782	17.3808	0.816031	0.710059	4362	26.33	38	28
33	1	448.498	430.14	172	1.7761	0.876419	14.7386	0.816731	0.632333	4888	28.0186	30	21
34	1	452.315	28.4134	130	1.30643	0.403502	12.0455	0.828571	0.787878	4559	50.4518	53	61
35	1	462.431	87.2254	142	2.70737	0.560366	13.4482	0.828103	0.788889	6157	43.2392	46	33
36	1	470.934	218.27	152	2.42376	0.92452	13.9114	0.824110	0.5	6163	27.2566	30	20
37	1	465.03	462.3	20	1.00187	0.382462	5.04627	0.469563	0.8	38	2.9	3	4
38	1	473.637	118.841	245	1.49331	0.763402	17.6419	0.835113	0.720388	5593	39.1551	39	30
39	1	479.337	235.395	152	1.89314	0.849103	17.6116	0.821217	0.466867	3655	37.2039	39	29
40	1	485.175	433.893	309	1.50055	0.745376	19.8331	0.830615	0.580571	11180	65.1605	48	35
41	1	488.297	19.5034	145	1.7151	0.812432	13.5875	0.800631	0.487321	4612	21.0089	33	22
42	1	52.114	81.2876	169	1.75774	0.822397	14.6889	0.8418	0.625926	3289	31.2959	33	24
43	1	21.8882	337.081	114	1.32358	0.456429	12.0478	0.808693	0.690508	4430	35.0331	41	20
44	1	26.5033	231.717	127	1.39677	0.498164	12.7142	0.813669	0.497802	4120	34.8031	35	23
45	1	33.697	138.233	121	1.44927	0.800701	12.8481	0.823077	0.6875	4555	36.5076	34	27
46	1	31.8729	216.532	111	1.64226	0.792324	11.8882	0.809838	0.770033	4359	39.2703	42	29
47	1	39.4234	413.723	222	1.78116	0.435105	16.8125	0.814401	0.687307	5624	43.3514	45	31
48	1	42.3592	301.33	103	1.85131	0.461364	11.4318	0.800342	0.47375	4366	42.3883	44	32
49	1	40.6712	471.441	222	1.69222	0.800486	16.8125	0.825	0.774224	3583	43.1667	41	33
50	1	46.594	35.3866	198	1.47235	0.732937	15.9377	0.821272	0.731618	5167	46.0633	48	37
51	1	32.8175	226.216	126	1.3288	0.461326	12.646	0.819708	0.763036	4550	36.1111	38	29
52	1	56.0102	81.1371	197	1.5958	0.506263	15.8376	0.831093	0.713549	8944	45.401	47	37
53	1	55.2709	265.187	176	1.09187	0.407481	13.0819	0.830346	0.734244	4700	35.0718	37	28
54	1	58.7305	465.979	141	1.44864	0.722373	13.3388	0.827632	0.471429	4518	32.0194	31	25
55	1	59.9231	102.546	130	1.06579	0.36589	17.8655	0.815493	0.769721	4593	35.3308	37	29
56	1	59.8505	253.991	107	1.52309	0.754275	11.672	0.84238	0.831326	4346	40.6168	43	33
57	1	77.2771	393.892	164	1.60733	0.49376	14.5181	0.848531	0.794238	4121	48.9217	52	37
58	1	75.5	213	106	1.52933	0.75667	11.6774	0.880303	0.880312	4366	41.1887	42	31
59	1	81.3667	139.839	180	1.37201	0.484467	15.1388	0.823077	0.714286	4187	47.15	49	37
60	1	78.683	245.397	142	1.7102	0.813229	13.4462	0.822078	0.759358	4839	32.669	33	25
61	1	90.7353	114.422	102	1.17952	0.530312	11.3361	0.810714	0.772727	4258	41.7131	45	34
62	1	85.9789	324.055	95	1.41567	0.707432	10.9981	0.804362	0.644326	4181	44.0105	45	36
63	1	89.9314	745.229	144	1.57597	0.712899	13.5106	0.817197	0.495714	4651	32.5744	34	26
64	1	89.4218	469.204	147	1.57767	0.759468	13.4908	0.807407	0.45423	4710	32.449	35	25
65	1	122.464	39.3708	178	1.24109	0.594028	15.0345	0.841799	0.781667	9293	32.2039	36	42
66	1	127.234	216.16	116	1.10719	0.429255	12.0478	0.828639	0.730769	4009	38.6734	40	21
67	1	113.248	122.979	233	2.3101	0.903304	13.274	0.806515	0.537416	9711	41.4781	43	34
68	1	103.283	178.186	144	1.42193	0.710922	13.5106	0.823077	0.75	5104	38.9187	41	32
69	1	190.918	79.4602	113	1.29241	0.40203	11.9348	0.818639	0.733766	4931	35.2124	40	21
70	1	193.423	196.613	188	1.45326	0.758882	14.8255	0.813023	0.45425	8079	48.0893	31	38
71	1	197.194	360.657	108	1.42853	0.711219	11.7265	0.815284	0.711429	4314	40.2222	41	31
72	1	205.418	335.408	97	1.52037	0.756993	11.1132	0.846011	0.621795	4188	42.1752	41	34
73	1	210.041	95.5238	168	1.67721	0.802814	13.7273	0.866228	0.449893	4888	31.7432	32	28
74	1	211.983	23.4	180	1.8442	0.807511	15.1368	0.813706	0.492308	8908	49.4889	35	60
75	1	217.202	73.9248	133	1.41276	0.83995	13.0131	0.83007	0.711323	4560	34.2837	35	24
76	1	217.053	255.215	189	1.7732	0.81624	19.5333	0.810581	0.465914	25016	42.3591	44	32

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1	3	216.083	64.7186	334	1.70565	0.810104	20.6219	0.89592	0.703158	15694	46.986	19	33	33
1	3	216.431	65.89	345	1.73559	0.712771	13.5973	0.917322	0.691155	4809	32.3379	23	23	33
1	3	216.783	67.0632	356	1.76553	0.725714	16.8415	0.905759	0.684509	9292	53.711	56	64	64
1	3	217.135	68.2364	367	1.79547	0.810627	13.7723	0.907978	0.681603	4158	61.6081	44	32	32
1	3	217.487	69.4096	378	1.82541	0.806796	15.319	0.923233	0.615023	7714	41.4731	44	34	34
1	3	217.839	70.5828	389	1.85535	0.801018	19.1492	0.857143	0.626087	12451	43.2326	45	32	32
1	3	218.191	71.756	400	1.88529	0.798253	13.9116	0.91010	0.703701	8165	61.875	44	34	34
1	3	218.543	72.9292	411	1.91523	0.832819	12.5102	0.91791	0.603922	4186	36.4715	36	29	29
1	3	218.895	74.1024	422	1.94517	0.805009	13.6103	0.916239	0.737374	5724	59.2035	40	30	30
1	3	219.247	75.2756	433	1.97511	0.802031	12.4634	0.917293	0.797186	4889	36.7851	39	30	30
1	3	219.599	76.4488	444	1.95525	0.853216	17.9316	0.830065	0.574661	10011	59.6528	42	31	31
1	3	219.951	77.622	455	1.97516	0.821266	12.9119	0.80726	0.660367	7879	60.145	63	48	48
1	3	220.303	78.7954	466	1.98531	0.772318	22.2123	0.807831	0.55698	16743	47.9309	47	36	36
1	3	220.655	79.9686	477	2.01525	0.847704	13.4933	0.910828	0.752632	4853	52.5385	34	27	27
1	3	221.007	81.1418	488	2.04519	0.802357	16.4865	0.913946	0.756366	5183	23.7123	24	19	19
1	3	221.359	82.315	499	2.07513	0.706031	14.8812	0.935484	0.725	6167	21.592	27	30	30
1	3	221.711	83.4882	510	2.10507	0.848032	17.367	0.939271	0.659091	5191	22.375	24	18	18
1	3	222.063	84.6614	521	2.13501	0.795621	18.3487	0.862188	0.752841	11566	43.5623	45	36	36
1	3	222.415	85.8346	532	2.16495	0.817466	17.0382	0.819255	0.632323	9439	41.3981	42	32	32
1	3	222.767	87.0078	543	2.19489	0.705349	16.4492	0.903226	0.717168	9210	43.2391	44	32	32
1	3	223.119	88.181	554	2.22483	0.634622	13.8198	0.943296	0.76123	4677	32.9123	34	27	27
1	3	223.471	89.3542	565	2.25477	0.492722	13.9116	0.921212	0.77551	4803	31.625	32	26	26
1	3	223.823	90.5274	576	2.28471	0.726189	12.1003	0.912688	0.737179	4837	38.5826	40	30	30
1	3	224.175	91.7006	587	2.31465	0.811294	12.4033	0.896632	0.738889	9883	37.1541	39	29	29
1	3	224.527	92.8738	598	2.34459	0.872617	17.1127	0.916335	0.775	8261	36.0043	38	28	28
1	3	224.879	94.047	609	2.37453	0.80372	16.0373	0.935195	0.765152	8567	42.4109	45	34	34
1	3	225.231	95.2202	620	2.40447	0.820613	13.3034	0.904937	0.820536	4291	30.8705	32	25	25
1	3	225.583	96.3934	631	2.43441	0.708752	16.1595	0.945235	0.770831	9626	37.166	38	30	30
1	3	225.935	97.5666	642	2.46435	0.834508	20.4158	0.81592	0.585714	10429	31.7957	35	25	25
1	3	226.287	98.7398	653	2.49429	0.718624	21.2003	0.822846	0.613913	12182	34.5099	36	25	25
1	3	226.639	99.913	664	2.52423	0.481118	16.0373	0.897778	0.701389	9021	41.6386	47	35	35
1	3	226.991	101.086	675	2.55417	0.811882	18.0893	0.855532	0.694721	9329	36.2996	38	27	27
1	3	227.343	102.259	686	2.58411	0.778004	13.359	0.93517	0.708313	4249	31.3897	33	25	25
1	3	227.695	103.432	697	2.61405	0.637235	15.3477	0.90508	0.608456	15605	52.398	55	40	40
1	3	228.047	104.605	708	2.64399	0.718833	20.3174	0.81573	0.686316	19203	55.0374	58	45	45
1	3	228.399	105.778	719	2.67393	0.85575	13.0358	0.90566	0.623377	3616	37.6661	39	29	29
1	3	228.751	106.951	730	2.70387	0.827833	17.8189	0.927391	0.717024	2386	37.3941	39	30	30
1	3	229.103	108.124	741	2.73381	0.805076	11.8802	0.90836	0.710719	4120	37.1171	39	28	28
1	3	229.455	109.297	752	2.76375	0.843339	14.6355	0.903108	0.658824	5693	33.8468	36	26	26
1	3	229.807	110.47	763	2.79369	0.621246	13.6103	0.925936	0.718718	4102	30.1507	32	25	25
1	3	230.159	111.643	774	2.82363	0.392803	14.1433	0.923977	0.752311	4223	39.3861	43	31	31
1	3	230.511	112.816	785	2.85357	0.742816	11.0382	0.926829	0.705882	3761	42.8134	45	31	31
1	3	230.863	113.989	796	2.88351	0.649328	11.9908	0.916699	0.723766	4218	37.3263	38	30	30
1	3	231.215	115.162	807	2.91345	0.878297	12.666	0.926431	0.612037	4172	33.1111	35	27	27
1	3	231.567	116.335	818	2.94339	0.717738	12.5651	0.91551	0.632653	4320	35.7258	37	28	28
1	3	231.919	117.508	829	2.97333	0.820261	12.5651	0.925373	0.810458	4449	35.079	37	31	31
1	3	232.271	118.681	840	2.99327	0.615031	11.9416	0.896	0.717919	4240	38.0357	40	29	29
1	3	232.623	119.854	851	3.02321	0.82417	15.7165	0.889908	0.777161	5114	27.9175	30	20	20
1	3	232.975	121.027	862	3.05315	0.80818	12.153	0.920635	0.753247	4190	38.7089	39	29	29
1	3	233.327	122.2	873	3.08309	0.55957	12.2033	0.910892	0.682308	3322	33.3714	34	26	26
1	3	233.679	123.373	884	3.11303	0.76782	13.4652	0.93211	0.759358	4693	33.0493	35	26	26
1	3	234.031	124.546	895	3.14297	0.274128	16.7366	0.92137	0.718954	4897	39.3318	41	31	31
1	3	234.383	125.719	906	3.17291	0.84626	25.7081	0.798679	0.567013	21853	49.8036	46	36	36
1	3	234.735	126.892	917	3.20285	0.590105	13.9116	0.915669	0.730769	4796	31.5526	32	25	25
1	3	235.087	128.065	928	3.23279	0.878875	15.6716	0.890993	0.680247	4827	43.8883	46	36	36
1	3	235.439	129.238	939	3.26273	0.878875	15.6716	0.890993	0.680247	4827	43.8883	46	36	36
1	3	235.791	130.411	950	3.29267	0.719387	11.3981	0.88493	0.728371	4113	42.1215	44	32	32
1	3	236.143	131.584	961	3.32261	0.45869	20.2166	0.852229	0.653223	16634	40.3235	42	31	31
1	3	236.495	132.757	972	3.35255	0.853532	13.4935	0.926571	0.722222	4322	29.5844	31	24	24
1	3	236.847	133.93	983	3.38249	0.746232	14.5381	0.937853	0.751131	1349	24.1988	27	20	20
1	3	237.199	135.103	994	3.41243	0.697748	15.8316	0.938095	0.781716	8672	41.0203	45	34	34
1	3	237.551	136.276	1005	3.44237	0.388889	17.2535	0.921176	0.758142	4182	30.3043	32	23	23
1	3	237.903	137.449	1016	3.47231	0.70512	12.2573	0.921878	0.784667	4250	36.0169	37	29	29
1	3	238.255	138.622	1027	3.50225	0.872327	20.0385	0.890148	0.681024	15023	47.5791	46	34	34
1	3	238.607	139.795	1038	3.53219	0.553923	13.3512	0.927132	0.777774	4609	31.4929	33	26	26
1	3	238.959	140.968	1049	3.56213	0.741888	12.3032	0.923183	0.721212	4241	35.1067	37	28	28
1	3	239.311	142.141	1060	3.59207	0.433552	12.1003	0.917463	0.763314	4179	37.3933	34	25	25
1	3	239.663	143.314	1071	3.62201	0.60562	12.1003	0.917463	0.763314	4179	37.3933	34	25	25
1	3	239.965	144.487	1082	3.65195	0.858893	9.50789	0.921078	0.764353	4086	33.3678	36	28	28
1	3	240.317	145.66	1093	3.68189	0.65998	14.1386	0.921078	0.764353	4086	102	100	82	82
1	3	240.669	146.833	1104	3.71183	0.645047	12.6157	0.919136	0.757316	4357	39.636	42	32	32
1	3	241.021	148.006	1115	3.74177	0.840367	11.3619	0.905028	0.736364	4389	41.023	41	28	28
1	3	241.373	149.179	1126	3.77171	0.823817	11.1132	0.898146	0.621155	3217	27.0826	28	24	24
1	3	241.725	150.352	1137	3.80165	0.807261	12.2409	0.795319	0.65	8432	36.0342	37	28	28
1	3	242.077	151.525	1148	3.83159	0.80431	17.3712	0.829612	0.593893	8025	38.0802	39	30	30

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1	30	132.516	470.016	250	1.71834	0.813219	17.0412	0.929368	0.631895	5098	20.392	21	16	23
2	31	126.67	118.226	115	1.26792	0.602239	12.1003	0.891673	0.6191	4361	26.1026	28	26	41
3	32	133.392	331.218	133	1.70893	0.810918	12.0573	0.910714	0.701333	4400	28.7382	29	22	33
4	33	129.828	361.081	99	1.31348	0.750701	11.2272	0.9	0.692308	4248	43.1111	43	34	51
5	34	135.862	376.305	186	1.48408	0.704616	15.7973	0.806584	0.640523	3241	47.118	49	37	59
6	35	136.281	415.62	171	1.40258	0.781603	18.7355	0.905574	0.678371	3972	23.2181	24	18	28
7	36	133.941	132	118	1.46352	0.641191	12.3373	0.914729	0.670455	4024	34.1011	26	26	42
8	37	163.395	60.8058	203	2.45822	0.913523	16.1359	0.923423	0.716783	4596	22.4195	24	17	24
9	38	134.271	497.208	107	2.11388	0.923925	11.472	0.89516	0.629412	4016	37.5727	38	28	47
10	39	141.448	240.271	181	1.38118	0.689916	15.1808	0.918182	0.709801	4158	44.7393	47	37	57
11	40	151.382	90.8072	181	1.20538	0.562378	15.1808	0.872999	0.716051	4163	45.0994	47	37	55
12	41	153.006	130.468	173	1.43106	0.791716	16.4435	0.910326	0.720833	4600	44.2773	45	33	53
13	42	154.19	371.018	103	1.51167	0.771169	11.5624	0.9275	0.717778	4529	43.1331	45	33	50
14	43	158.17	311.535	159	1.82167	0.935858	16.2287	0.913793	0.722727	4653	28.0063	29	21	33
15	44	163.846	62.5155	77	1.30584	0.612091	11.132	0.92381	0.746154	4093	42.1753	45	33	50
16	45	163.826	197.375	73	1.19563	0.517937	9.40888	0.879518	0.643635	3333	31.9389	32	24	39
17	46	164.439	463.571	103	1.30539	0.612714	11.5624	0.905172	0.731265	4096	39.0035	40	30	48
18	47	169.289	73.1358	81	1.09686	0.410546	10.1554	0.89011	0.710364	3980	49.1358	50	40	60
19	48	171.28	161.191	93	1.49721	0.728516	10.8817	0.91	0.701545	4151	37.1075	39	30	43
20	49	171.294	405.46	126	1.15275	0.697403	12.466	0.933333	0.807632	3872	30.7302	32	24	37
21	50	171.661	492.472	328	1.81566	0.815987	12.1662	0.95105	0.621151	4145	32.3288	34	23	40
22	51	173.47	222.276	203	1.35588	0.673379	14.0169	0.911193	0.751832	3986	44.266	46	34	53
23	52	178.902	433.335	102	1.81242	0.83103	11.3961	0.91931	0.60355	4289	42.019	43	31	54
24	53	183.608	66.8987	727	2.75657	0.931781	17.3712	0.922612	0.50441	4974	37.465	41	29	47
25	54	194.827	300.464	182	1.61442	0.834612	16.3619	0.926616	0.73512	4657	27.5123	28	21	34
26	55	196.489	327.523	282	1.83588	0.842417	18.3687	0.98135	0.581645	4826	24.841	37	28	42
27	56	193.333	198.188	69	1.37759	0.687739	9.37202	0.907895	0.784091	2189	31.7266	33	24	35
28	57	197.394	480.866	182	1.34735	0.693164	13.4662	0.910356	0.642693	4317	30.4014	31	24	37
29	58	198.895	14.0369	172	1.52588	0.759398	14.7986	0.919386	0.671873	4151	49.1331	51	40	60
30	59	203.48	182.922	103	1.19441	0.616631	11.4316	0.927928	0.780203	4291	41.4602	44	34	50
31	60	207.9	141.831	90	1.15049	0.694073	10.7047	0.909051	0.743803	4030	44.7378	47	35	54
32	61	206.535	131.591	127	1.13094	0.647075	12.7162	0.894366	0.67802	4249	33.6587	36	23	33
33	62	211.352	442.087	127	1.19316	0.762352	12.7162	0.901183	0.721591	4210	33.2124	35	26	40
34	63	214.444	54.7969	192	1.19282	0.5147	13.4353	0.923077	0.703882	4632	45.0625	48	35	57
35	64	217.839	201.412	118	1.21036	0.563371	13.7173	0.930148	0.704762	4994	47.2368	50	39	57
36	65	216.688	175.738	166	1.70375	0.809229	16.4303	0.922318	0.784889	3629	34.2823	36	28	40
37	66	218.676	384.658	204	2.15034	0.885388	14.1165	0.910314	0.63354	4637	22.7384	24	18	27
38	67	220.865	350.827	104	1.29582	0.635975	11.5073	0.924571	0.722222	4228	40.6338	42	31	49
39	68	229.603	236.64	116	1.82958	0.714826	13.159	0.977119	0.653846	3847	28.0221	30	22	36
40	69	229.632	467.224	106	1.38212	0.690291	11.4174	0.930356	0.688312	4010	37.8302	39	31	46
41	70	240.147	249.048	143	2.56119	0.92085	13.4935	0.93295	0.929335	4059	28.7846	29	21	37
42	71	236.88	341.882	87	1.61995	0.709952	10.28	0.882919	0.703102	4079	49.1464	50	38	61
43	72	244.699	88.3706	143	1.38306	0.690813	13.4935	0.916667	0.744732	4294	30.029	31	27	40
44	73	251.332	18.672	123	1.8051	0.556056	12.4157	0.905397	0.744048	4150	33.2	34	27	40
45	74	253.463	309.288	80	1.62102	0.709952	10.0923	0.909051	0.740741	3944	48.2	51	33	61
46	75	257.025	44.1698	158	1.56254	0.76839	14.2183	0.929825	0.80303	4305	27.0755	28	21	33
47	76	259.014	278.679	140	2.4612	0.823935	13.3312	0.915073	0.675	4146	28.6113	31	22	37
48	77	252.388	332.233	87	1.40256	0.701183	10.5748	0.887755	0.725	4350	50.6598	51	38	63
49	78	258.629	147.266	124	1.03654	0.683166	12.5631	0.911765	0.733728	4215	33.9819	36	26	41
50	79	269.735	287.283	113	1.35841	0.674824	11.9948	0.904	0.732766	3856	34.1239	35	26	42
51	80	273.247	97.7288	118	1.47981	0.731131	12.2573	0.880597	0.610332	3883	32.7582	35	26	42
52	81	271.644	129.856	118	1.90244	0.850706	12.2573	0.93939	0.670455	3988	32.7966	35	26	41
53	82	281.493	495.373	236	2.40848	0.903166	18.4134	0.902232	0.560104	18228	41.5811	61	68	74
54	83	288.773	400.937	267	2.9475	0.937733	21.4166	0.882232	0.50551	14136	38.5377	40	31	47
55	84	280.18	201.696	123	1.10317	0.432421	12.4157	0.932836	0.801282	4211	33.648	35	25	41
56	85	289.615	423.563	96	1.49853	0.741771	11.0558	0.90346	0.738462	4561	47.5104	50	37	54
57	86	292.616	475.965	66	1.19599	0.548328	9.167	0.90411	0.733333	7232	109.376	132	84	134
58	87	299.154	390.016	128	2.90388	0.916924	12.666	0.9	0.734642	4321	34.773	35	26	43
59	88	304.173	264.408	301	1.1756	0.525765	19.5766	0.847887	0.651515	16051	59.9101	61	47	71
60	89	301.379	159.345	87	1.32239	0.654235	10.5748	0.90625	0.725	3930	45.1721	46	33	57
61	90	306.448	429.132	112	1.39275	0.656038	11.9118	0.903228	0.717949	4786	42.7321	45	33	51
62	91	305.333	335.758	33	1.52246	0.754039	8.1804	0.91467	0.63469	5488	166.303	170	138	199
63	92	310.047	197.459	128	1.61717	0.831161	12.7862	0.914286	0.809324	4232	33.0623	35	26	39
64	93	310.445	88.1628	129	1.39276	0.575751	12.8159	0.889655	0.661536	4033	31.1636	33	24	37
65	94	319.984	169.903	126	1.25066	0.63271	12.5451	0.925373	0.733728	4261	16.3629	35	24	42
66	95	315.932	374.111	235	1.6	0.83179	17.2777	0.915197	0.658263	8844	37.6126	39	29	67
67	96	316.101	433.826	87	1.30457	0.612359	10.5748	0.913789	0.725	4236	48.4897	51	41	55
68	97	325.081	416.453	66	1.70754	0.810574	10.4462	0.931783	0.816523	4283	49.9023	51	42	59
69	98	331.918	24.1634	243	2.42612	0.910948	17.5897	0.815438	0.54	8882	16.5516	37	27	45
70	99	331.821	21.5464	312	1.38432	0.692517	11.5416	0.918033	0.666667	1149	27.0446	38	30	43
71	100	332.441	151.076	188	1.34318	0.674607	12.7182	0.907801	0.711111	7759	60.6172	62	49	74
72	101	335.986	222.69	162	1.72274	0.814295	13.4662	0.934211	0.746889	4547	32.7256	34	26	38
73	102	350.436	153.236	130	1.92529	0.697386	11.6365	0.901639	0.714786	4340	39.4543	40	30	47
74	103	354.173	470.723	126	1.72219	0.811689	12.7662	0.927536	0.711111	4253	33.2246	33	23	41
75	104	356.548	436.774	287	1.67429	0.80204	18.6221	0.932404	0.738741	9779	42.7608	44	34	53
76	105	357.323	232.198	118	1.22431	0.576819	12.2573	0.916729	0.702381	4199	35.5817	37	28	44

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1	6	359.018	312.36	116	1.78064	0.827612	12.0478	0.597639	0.426374	4181	36.4754	38	43
1	6	361.764	359.031	129	1.60135	0.781088	12.8159	0.508451	0.461528	7326	36.4166	40	71
1	6	370.42	326.746	117	1.127866	0.44267	11.5416	0.503276	0.371889	4218	37.4607	40	66
1	6	379.866	301.359	97	1.21577	0.568377	11.1132	0.491188	0.371888	4114	42.7216	41	31
1	6	382.727	401.684	187	1.74828	0.820259	15.4704	0.571793	0.611111	8130	48.1189	41	31
1	6	388.96	393.188	103	1.87034	0.835632	19.4414	0.507186	0.611111	11210	31.9143	39	43
1	6	397.153	337.23	174	1.75594	0.821939	14.8813	0.513399	0.775	8757	50.3316	37	81
1	6	398.868	261.411	136	1.86261	0.804732	13.159	0.591137	0.633816	4377	32.8369	35	41
1	6	401.478	202.432	134	1.51597	0.733039	12.8591	0.44109	0.613101	11681	51.5139	30	41
1	6	399.517	327.516	169	2.07819	0.872445	13.7736	0.725166	0.637327	5111	36.1156	38	44
1	6	416.493	373.039	384	1.50902	0.718901	12.1114	0.629371	0.601881	9953	38.0186	31	37
1	6	426.434	70.7842	159	1.6318	0.790105	14.2203	0.510928	0.60103	4397	38.0186	31	37
1	6	429	14	109	1.747376	0.536169	11.7806	0.531824	0.712234	3103	35.8073	37	17
1	6	436.508	174.755	132	1.18234	0.690166	12.9611	0.523077	0.713333	4152	31.4345	33	38
1	6	442.816	371.7	395	1.84433	0.80664	22.1211	0.531807	0.801217	10717	25.8438	74	31
1	6	435.618	108.709	91	1.82124	0.713351	10.1611	0.51	0.717378	3761	61.3176	43	31
1	6	436.969	245.641	131	1.71057	0.818408	12.9119	0.591156	0.727778	4781	32.7023	34	39
1	6	446.315	472.393	110	1.52412	0.735407	11.9315	0.52327	0.715714	4289	38.8091	40	47
1	6	447.594	87.8949	178	1.58878	0.735618	13.0515	0.52728	0.659172	4181	35.1697	27	30
1	6	450.265	488.54	113	1.77801	0.81812	12.9918	0.541667	0.807143	4353	34.8106	39	40
1	6	456.785	110.913	107	1.25762	0.606412	11.7172	0.50878	0.718252	3188	36.3364	38	44
1	6	456.12	359.5	100	1.27419	0.620241	11.2938	0.525826	0.769231	3058	38.58	41	47
1	6	481.271	112.624	123	1.7004	0.808793	13.0131	0.575	0.53275	4167	31.7972	33	38
1	6	482.38	188.899	257	1.7078	0.810437	18.0993	0.571310	0.673891	9420	38.6537	30	46
1	6	472.762	297.529	101	1.12911	0.644334	11.3101	0.50931	0.765152	9080	80	79	38
1	6	471.607	328.426	145	1.73853	0.589322	11.5875	0.523567	0.739394	4309	28.7172	30	38
1	6	471.513	422.586	118	1.10393	0.423593	17.1557	0.513386	0.76359	4237	36.5259	30	43
1	6	478.43	272.1851	109	1.42015	0.710159	11.7806	0.523728	0.718571	4370	38.1743	40	31
1	6	485.604	60.2023	164	2.07652	0.816405	14.4503	0.512031	0.780952	5814	30.1561	33	37
1	6	490.38	256.707	178	1.15137	0.707318	8.95423	0.55351	0.65	8763	30.7349	109	85
1	6	497.006	313.487	62	1.60843	0.782231	8.88467	0.595531	0.605195	3893	39.3218	41	47
1	6	496.029	311.5	209	2.09018	0.878165	16.2737	0.521136	0.521303	8882	82.7019	43	31
1	6	480.719	7.92708	96	1.10594	0.477029	11.0558	0.50566	0.727773	1030	41.9782	44	37
1	6	498.817	358.104	115	1.18889	0.531714	12.1005	0.542623	0.804196	4250	36.4585	38	44
1	6	501.108	502.892	93	1.60155	0.553584	10.8217	0.520732	0.780593	3878	41.6989	42	32
1	6	512.717	58.4345	99	1.62754	0.713654	11.2727	0.51882	0.707143	3940	38.798	40	32
1	6	522.857	88.0386	98	1.39204	0.655462	11.1704	0.589099	0.7	3920	40.1827	42	32
1	6	526.348	245.156	96	1.19415	0.546597	11.0558	0.55077	0.727773	4129	43.0104	44	34
1	6	512.359	73.5351	271	1.67368	0.801381	18.5355	0.573237	0.534589	8189	31.7247	32	34
1	6	517.97	130.52	100	1.30965	0.665768	11.2838	0.525918	0.769231	4241	42.41	44	34
1	6	50.458	144.628	131	1.82018	0.835462	11.9119	0.503618	0.727778	3658	43.1908	44	34
1	6	50.0866	486.481	116	1.49292	0.715017	12.1533	0.513336	0.686391	4109	35.4724	37	28
1	6	58.7195	153.728	221	1.71096	0.811418	16.7746	0.528571	0.701587	3177	23.4253	24	18
1	6	55.513	379.207	114	1.15451	0.502746	12.1005	0.505512	0.737179	4271	37.1813	38	29
1	6	55.7714	414.6	35	1.37355	0.687772	6.97558	0.574736	0.729167	3401	15.4	166	120
1	6	66.23	272.97	100	1.08978	0.571077	11.7639	0.517431	0.755716	4182	41.82	43	34
1	6	49.9759	74.6717	166	1.23086	0.583018	11.5381	0.517127	0.741071	8273	49.8773	32	40
1	6	49.7219	37.807	151	1.40031	0.727228	13.8658	0.520732	0.719048	5049	33.4371	35	24
1	6	53.4802	91.3282	133	1.67867	0.803164	13.5573	0.510716	0.727277	3574	56.0332	38	41
1	6	58.2083	30.3333	96	1.58243	0.768118	11.0558	0.497196	0.664667	3591	37.4063	40	30
1	6	66.7215	223.744	219	1.83108	0.817143	14.4985	0.539914	0.711038	4899	22.8265	24	18
1	6	68.2202	889.317	109	1.93194	0.8561	11.7806	0.572	0.605556	4065	31.2836	38	28
1	6	77.1403	261.287	108	1.48831	0.732245	11.7265	0.515234	0.72	4321	40.0093	42	33
1	6	79.8741	132.219	125	1.65213	0.798019	12.1106	0.5	0.648857	4592	34.0116	36	26
1	6	78.1333	311.013	75	1.25902	0.607157	9.77205	0.514634	0.757578	3898	51.9733	33	42
1	6	79.1619	422.848	138	1.26926	0.61505	13.3555	0.524174	0.764667	4137	32.1522	33	25
1	6	84.9439	277.058	156	2.2151	0.884372	14.0325	0.528571	0.723297	4899	30.1218	31	37
1	6	85.1039	369.268	153	1.70677	0.810381	13.9573	0.516108	0.732057	4526	28.5817	30	37
1	6	91.7971	42.0638	243	1.77297	0.825758	20.9581	0.5425	0.709877	3207	31.9333	38	30
1	6	86.2016	321.219	73	1.28553	0.628358	9.48888	0.535887	0.727274	3957	56.2053	55	43
1	6	95.0667	101.005	193	1.76841	0.874763	19.757	0.542029	0.77381	9648	49.8785	52	41
1	6	94.8165	187.977	178	1.5115	0.781661	16.8813	0.55082	0.78323	4673	26.4563	28	31
1	6	95.9316	388	122	1.57166	0.772163	12.9611	0.591892	0.628571	4167	33.8408	38	24
1	6	96.0947	287.728	111	1.36669	0.693664	12.0178	0.597638	0.74026	4079	35.7987	36	28
1	6	104.786	46.024	187	2.0048	0.864715	14.5819	0.522652	0.723244	3204	55.1138	57	43
1	6	100.91	217.72	100	1.32022	0.659142	11.2838	0.517431	0.749231	4252	42.52	43	33
1	6	105.819	482.487	159	1.88554	0.847777	16.2263	0.503409	0.571881	4668	29.3385	30	23
1	6	108.051	181.037	136	1.77393	0.825944	13.159	0.519919	0.8	4216	31.0167	31	23
1	6	114.3	219.5	148	1.5081	0.745397	12.7273	0.51358	0.560716	4563	30.8311	32	39
1	6	112.944	348.88	125	1.12674	0.66078	12.8157	0.512109	0.741071	4232	33.836	34	27
1	6	112.696	399.301	159	2.35954	0.967239	21.3797	0.5975	0.543939	21578	60.1059	60	43
1	6	118.715	407.016	123	1.96567	0.860824	12.3143	0.511111	0.583716	4530	36.8293	38	29
1	6	125.698	191.671	148	1.82258	0.836838	13.7736	0.508337	0.712919	4381	28.4618	30	24
1	6	127.837	335.779	190	1.73451	0.817074	15.5538	0.592019	0.69871	4777	25.1421	26	20
1	6	119.657	269.457	105	1.25891	0.638191	11.5636	0.513013	0.728167	3997	38.0667	40	47

1	1	132.821	116.065	116	1.37911	0.77403	12.2373	0.395131	0.3375	1370	37.0339	39	29	31
2	1	131.089	119.464	192	1.65987	0.81191	15.6353	0.81866	0.627451	4755	24.7656	29	19	31
3	1	144.273	127.203	290	1.81173	0.832835	18.4788	0.82204	0.325572	9310	31.9128	33	23	33
4	1	145.757	132.468	141	1.1513	0.491674	13.3988	0.915586	0.723077	1314	30.3537	32	24	37
5	1	148.67	136.2611	203	1.35381	0.671093	16.0769	0.810188	0.743158	9182	45.7315	27	35	55
6	1	151.921	139.331	189	1.68864	0.80503	19.1326	0.935644	0.75	4303	33.9418	26	30	32
7	1	150.219	135.6716	160	1.91884	0.853632	16.6609	0.923197	0.470833	4619	27.5088	28	21	31
8	1	155.972	145.497	187	2.03862	0.871425	15.1304	0.921182	0.876054	7571	40.0866	32	21	68
9	1	155.378	139.718	335	1.54998	0.74604	13.1106	0.912182	0.767015	4282	31.7926	33	25	39
10	1	166.223	188.369	158	2.46016	0.911683	15.8777	0.91093	0.6875	3235	26.4394	27	20	32
11	1	177.089	301.02	326	3.04239	0.94161	20.6835	0.933821	0.729482	5938	27.2788	31	21	37
12	1	175.35	180.975	160	1.74316	0.81923	14.273	0.91954	0.63761	4176	27.975	29	22	31
13	1	182.463	312.893	177	2.04497	0.874919	15.0121	0.907692	0.578431	6096	16.4107	34	27	61
14	1	187.062	31.94882	193	1.33599	0.471632	15.6759	0.818279	0.709539	8598	43.828	26	34	53
15	1	183.867	145.944	143	1.88217	0.804117	13.4333	0.89371	0.617059	4378	30.7437	31	24	37
16	1	187.435	426.351	331	1.32017	0.653061	12.9149	0.933716	0.719762	19335	33.1527	34	26	39
17	1	181.717	234.087	388	1.4823	0.804139	22.2245	0.84143	0.61667	19335	49.8225	32	39	60
18	1	187.864	466.18	152	1.43687	0.791721	13.9116	0.921212	0.715098	4421	29.0553	30	22	32
19	1	191.776	331.251	208	2.18997	0.881751	16.2131	0.920334	0.630303	8950	63.0288	21	33	52
20	1	199.537	31.6239	147	2.09227	0.8781	13.4609	0.924328	0.8125	4256	28.9524	30	23	31
21	1	199.265	391.486	326	1.16378	0.511034	16.9833	0.923864	0.718582	8913	39.4381	42	30	48
22	1	202.494	117.496	171	1.8046	0.832822	12.4122	0.882312	0.417059	4288	35.3207	28	27	44
23	1	223.218	471.515	356	1.32249	0.754197	11.0935	0.804517	0.461438	4263	27.2397	37	21	33
24	1	229.645	75.8135	110	1.42258	0.801567	11.8345	0.82437	0.727373	6320	51.4545	41	46	69
25	1	225.35	217.611	206	1.72215	0.81138	14.1853	0.923767	0.754579	4394	21.3201	22	16	27
26	1	230.39	311.381	160	2.02402	0.869124	16.273	0.930233	0.8	7533	47.2042	50	37	51
27	1	229.234	47.3761	117	1.78922	0.823234	12.2053	0.9	0.64773	6538	55.8803	59	44	67
28	1	230.232	426.411	183	1.30452	0.747143	13.4835	0.916667	0.480932	4484	31.3566	32	23	38
29	1	233.37	135.318	127	1.19808	0.530763	12.7162	0.92039	0.785332	4318	31.2143	35	26	42
30	1	239.567	368.239	180	1.3281	0.820022	15.1388	0.923077	0.703125	9211	51.3289	53	40	61
31	1	245.931	256.323	213	1.81026	0.833575	17.3897	0.94186	0.718846	3636	33.1934	34	18	28
32	1	246.538	327.9	170	1.66474	0.799479	12.0855	0.921986	0.666647	4466	38.2	35	24	43
33	1	254.628	478.726	317	1.56468	0.746396	20.0902	0.948116	0.600379	14100	44.4795	44	32	57
34	1	251.756	330.701	127	1.30066	0.745619	12.7162	0.913469	0.47959	1629	34.4188	37	29	45
35	1	260.117	431.62	115	2.16747	0.882754	13.3875	0.935062	0.635965	4313	29.7418	30	24	37
36	1	272.667	226.453	283	2.07848	0.876654	19.0192	0.92233	0.633233	7284	25.591	26	20	31
37	1	271.121	89.2339	149	1.50153	0.747124	13.7136	0.91411	0.672208	4396	29.5034	30	22	37
38	1	271.065	418.278	123	1.4177	0.70884	12.5143	0.938931	0.727811	4288	36.8618	37	28	41
39	1	275.616	457.498	129	1.19877	0.551482	12.8159	0.920098	0.708791	4334	33.5869	35	26	41
40	1	281.035	107.674	116	1.34106	0.680115	13.5106	0.911176	0.8	6390	30.6861	32	23	34
41	1	283.372	378.191	180	1.37189	0.684397	15.1388	0.922682	0.75	8530	47.5464	49	37	59
42	1	281.019	205.957	208	1.35849	0.766881	16.2737	0.924444	0.718837	9450	45.4237	48	35	56
43	1	281.844	625.593	135	1.92388	0.854314	15.1106	0.944056	0.832323	4238	31.3926	32	25	38
44	1	302.061	695.628	164	1.18433	0.530012	14.4503	0.906077	0.689076	5196	33.5122	35	23	42
45	1	326.196	440.576	215	2.71841	0.923881	10.1723	0.657169	0.383956	20112	26.1287	29	21	35
46	1	318.028	319.6	180	1.88826	0.932348	15.1388	0.909091	0.625	8018	44.5411	46	34	55
47	1	319.139	460.525	122	1.19158	0.543794	12.4634	0.902704	0.72819	4028	32.0164	35	27	39
48	1	317.788	397.364	165	1.73580	0.817119	14.4943	0.901638	0.651762	4708	26.5212	30	23	34
49	1	316.806	170.802	191	2.02727	0.86974	15.5415	0.867718	0.60319	8694	45.5183	48	37	54
50	1	344.676	379.211	21	1.67836	0.803276	9.50789	0.910236	0.739593	4011	36.493	57	46	69
51	1	353.416	344.282	213	2.0815	0.774904	14.4682	0.918103	0.591667	8798	41.3032	42	32	50
52	1	362.19	54.9241	158	1.5869	0.774933	14.1325	0.913253	0.463866	4870	30.8228	32	24	38
53	1	362.172	320.46	87	1.04942	0.704104	10.5218	0.915789	0.719008	3703	42.5632	44	33	52
54	1	361.328	386.148	41	1.41665	0.707125	8.1392	0.844038	0.7425	3820	62.623	61	51	76
55	1	377.575	322.072	259	1.65988	0.800832	10.1395	0.813233	0.6475	13564	52.7558	51	39	67
56	1	380.455	482.861	231	2.63929	0.927763	17.1499	0.916667	0.712963	5920	33.7558	51	39	67
57	1	383.223	355.813	121	1.30432	0.62168	12.4122	0.916667	0.712963	5920	25.1548	26	18	31
58	1	398.118	460.118	161	2.14008	0.884113	11.3175	0.907603	0.557093	3264	32.5716	34	27	41
59	1	393.224	337.158	76	1.53277	0.765132	9.8358	0.907603	0.557093	3264	32.5716	34	27	41
60	1	400.168	234.395	203	1.63778	0.791934	16.0769	0.922727	0.457182	8751	43.1044	45	38	62
61	1	403.29	274.575	193	1.65013	0.81339	15.4759	0.923445	0.430719	6930	36.0104	37	27	44
62	1	403.127	361.542	142	1.23011	0.582232	13.4487	0.92078	0.728203	4316	30.6036	32	23	37
63	1	416.389	360.643	126	1.31237	0.647598	12.666	0.913043	0.75	4165	33.0556	34	26	40
64	1	423.782	437.089	436	1.87314	0.839134	23.5612	0.912	0.619318	18633	42.7362	44	33	53
65	1	423.267	58.6533	150	1.28276	0.627084	13.8138	0.91667	0.76125	4218	28.12	29	22	31
66	1	437.237	209.737	38	1.71276	0.811859	6.9558	0.821087	0.59375	3012	79.2632	80	41	100
67	1	442.313	200.837	35	1.44058	0.719817	6.97558	0.875	0.729167	2912	92.0571	82	40	100
68	1	440.931	222.468	174	1.35735	0.82231	11.8843	0.933486	0.750309	5908	33.954	35	27	40
69	1	443.41	277.19	105	1.78618	0.822653	11.5628	0.87436	0.425	3001	37.1524	38	30	45
70	1	446.314	371.648	122	1.43784	0.709713	12.4631	0.917293	0.71619	4353	37.382	39	30	45
71	1	473.447	121.924	119	1.70559	0.810088	12.3092	0.85013	0.610336	2884	33.473	35	26	40
72	1	482.814	239.798	114	1.13166	0.69339	12.0678	0.914126	0.737203	4288	37.0877	38	29	44
73	1	488.5	287.192	104	1.15514	0.600955	11.4073	0.816382	0.717273	3893	37.4377	38	29	44
74	1	497.774	121.27	133	1.44318	0.721103	12.1005	0.81673	0.49457	3220	36.087	38	27	41
75	1	499.628	314.244	127	1.47034	0.733067	12.7162	0.913669	0.705556	4456	31.9191	37	26	41
76	1	503.036	134.754	154	1.15883	0.503112	14.0835	0.919139	0.8	4764	30.4103	32	23	37

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1	1	241.36	45.3128	211	1.45795	0.408118	16.3907	0.546188	0.733571	8125	24.5515	90	67
1	1	241.66	308.241	191	1.40919	0.783169	15.5945	0.927184	0.772279	4521	23.6702	35	29
1	1	249.134	425.694	149	1.63414	0.790903	13.7736	0.92125	0.710246	4359	29.255	20	36
1	1	248.31	378.26	73	1.20745	0.597015	9.64088	0.890744	0.675926	3060	61.9178	43	51
1	1	248.378	398.218	156	2.20066	0.990793	14.0935	0.92459	0.6	9763	62.5433	59	80
1	1	252.588	265.29	102	1.81198	0.839801	13.2227	0.896552	0.594771	4283	22.323	39	19
1	1	273.356	401.522	231	2.31573	0.903716	17.9749	0.931126	0.518398	9000	35.8366	37	44
1	1	272.527	411.305	131	2.13733	0.884078	12.9149	0.99236	0.641416	4192	34.2901	34	26
1	1	272.769	485.615	143	1.31807	0.492149	12.4935	0.928511	0.744792	4196	28.1427	31	23
1	1	278.037	187.931	248	1.50594	0.7177	11.4728	0.930536	0.706935	8918	32.7181	35	40
1	1	278.313	457.212	150	1.60585	0.782163	12.9198	0.892206	0.884664	4322	28.8131	29	36
1	1	282.808	48.0113	177	1.31211	0.453231	15.0123	0.918189	0.784667	4236	23.322	25	20
1	1	292.118	61	170	1.75569	0.822163	14.7123	0.918918	0.722727	4143	24.3706	26	19
1	1	293.317	412.439	232	1.32813	0.455479	12.9441	0.916667	0.732333	4542	49.5406	50	39
1	1	300.019	211.943	105	1.43507	0.717238	11.5124	0.905132	0.673077	3901	31.1526	39	45
1	1	301.375	245.225	136	1.35237	0.672221	13.139	0.915307	0.747252	7747	57.1103	59	69
1	1	303.395	44.7277	244	1.92196	0.861881	16.734	0.718761	0.597285	8734	33.0831	34	40
1	1	308.638	83.2276	116	1.25302	0.402565	12.153	0.899233	0.617363	4893	55.4224	61	73
1	1	311.872	109.356	129	1.66416	0.72138	15.0367	0.921481	0.721856	6157	37.1899	38	46
1	1	319.228	247.71	272	1.72323	0.814731	18.8091	0.874598	0.618182	9284	34.0589	35	42
1	1	320.663	419.596	312	1.59125	0.778498	19.9311	0.891439	0.619048	18606	59.4346	62	72
1	1	322.679	198.71	296	1.77825	0.826468	19.4134	0.87571	0.770833	9157	31.9493	33	38
1	1	328.216	156.173	335	1.62431	0.768613	20.6327	0.856717	0.744144	14183	41.2328	44	53
1	1	328.84	116.43	241	2.40372	0.902234	17.6298	0.893773	0.675641	9558	39.1721	41	47
1	1	329.432	216.832	119	1.69567	0.803564	12.3092	0.901513	0.613816	4050	36.0326	35	41
1	1	332.686	380.245	102	1.34389	0.680061	11.3961	0.910716	0.728571	3971	28.9141	40	47
1	1	340.491	119.404	304	1.12748	0.456237	11.6174	0.921739	0.736111	4013	27.8385	39	46
1	1	348.5	126.445	110	1.50007	0.745305	11.8345	0.901639	0.454762	4017	36.3182	38	46
1	1	347.429	316.767	91	1.95173	0.848021	10.7461	0.919192	0.631946	3658	40.1978	41	50
1	1	348.016	238.455	171	1.16875	0.492147	12.4122	0.923664	0.715641	4263	35.2314	37	43
1	1	348.932	268.525	158	1.65608	0.797117	16.1835	0.908016	0.492982	4752	30.8759	32	37
1	1	352.05	489.95	119	1.59372	0.778647	12.3092	0.929688	0.704142	4086	18.2361	35	41
1	1	352.35	395.033	75	1.68499	0.840551	15.2227	0.892571	0.611076	3532	37.0933	49	60
1	1	354.688	111.082	182	1.12392	0.456455	15.1388	0.921835	0.75	8814	42.4505	44	53
1	1	360.267	237.789	180	1.72302	0.832527	11.5364	0.93735	0.807652	3915	37.2837	37	45
1	1	360.8	294.429	105	1.43774	0.69252	12.153	0.903496	0.70203	3995	31.3574	36	41
1	1	360.421	154.388	116	1.3587	0.476884	15.1388	0.910367	0.66667	4291	27.8385	24	16
1	1	368.133	45.8833	186	1.68104	0.805389	10.7047	0.909091	0.75	3718	41.2111	42	52
1	1	367.3	432.778	90	1.52378	0.754535	11.5073	0.913281	0.693333	3903	37.5188	38	44
1	1	365.721	494.077	104	1.71092	0.846601	10.7661	0.91	0.758233	3794	41.4923	42	51
1	1	368.154	214.296	91	1.34563	0.68102	12.2092	0.901513	0.708232	4159	34.9486	36	43
1	1	372.21	275.807	119	1.8509	0.30746	12.4935	0.914647	0.739592	4415	30.8741	33	38
1	1	373.35	213.65	143	2.12983	0.882919	17.1127	0.855019	0.413333	8527	37.0739	38	46
1	1	376.913	76.1678	230	1.34195	0.668537	12.9149	0.922535	0.71978	4152	27.9847	36	40
1	1	379.519	267.099	131	1.19238	0.548875	12.1005	0.927619	0.804196	4154	36.3063	38	43
1	1	385.722	271	115	1.18461	0.629051	16.5919	0.922961	0.735456	8466	50.058	52	60
1	1	388.102	93.1497	167	1.22361	0.574261	15.3061	0.937716	0.773105	8747	47.538	50	57
1	1	394.136	163.607	164	2.28349	0.907731	18.5091	0.883041	0.719018	17247	57.1755	58	70
1	1	401.325	17.596	202	2.69457	0.825864	19.9211	0.850136	0.484472	9922	31.8013	33	39
1	1	398.503	250.074	312	1.43899	0.719074	10.8817	0.896331	0.713385	3854	41.4109	42	52
1	1	398.398	391.467	93	2.83274	0.913661	17.0007	0.886719	0.493178	4413	19.4105	19	25
1	1	408.33	138.143	223	1.49831	0.744744	11.9231	0.935029	0.747863	4356	28.32	26	31
1	1	417.883	122.789	175	1.13504	0.673065	11.3961	0.910716	0.708233	4055	39.5588	41	48
1	1	419.559	405.314	102	1.84651	0.840283	17.2609	0.917368	0.782609	9125	38.9957	41	51
1	1	415.318	188.923	234	1.38902	0.777169	15.389	0.923732	0.775	8002	43.0215	44	51
1	1	414.946	88.2473	186	1.23599	0.587712	12.7162	0.927007	0.751479	3960	31.1811	33	38
1	1	417.828	270.362	127	1.12741	0.661785	9.8198	0.873363	0.690909	3362	41.2368	46	55
1	1	417.278	30.0789	76	1.62912	0.76946	16.9273	0.925924	0.747544	1897	45.1257	47	56
1	1	431.223	108.749	173	1.21582	0.649911	11.8345	0.916647	0.703128	4025	36.3505	38	45
1	1	431.809	422.808	110	1.51513	0.772428	14.4943	0.913016	0.705128	5011	30.5315	31	37
1	1	439.594	316.903	145	1.69167	0.818831	11.8882	0.840932	0.652911	3516	31.6757	33	39
1	1	441.477	119.946	111	1.30033	0.669309	9.05824	0.910716	0.609524	7017	138.176	110	165
1	1	442	394	51	2.94177	0.940157	27.3556	0.788005	0.541151	20652	51.5012	51	64
1	1	489.772	225.858	401	1.22476	0.65146	11.5626	0.873563	0.616571	3058	40.2288	41	49
1	1	475.079	114.763	76	1.77782	0.826005	9.83698	0.873563	0.616571	3058	32.5429	35	40
1	1	479.714	244.429	105	1.33337	0.65146	11.5626	0.873563	0.616571	3058	34.5137	35	42
1	1	482.952	495.032	146	1.66638	0.753924	13.4343	0.906032	0.717374	5039	42.5437	44	52
1	1	487.34	149.102	197	1.83365	0.878202	15.8376	0.907834	0.643191	8102	22.0571	32	40
1	1	493.055	44.1978	91	1.37016	0.486216	10.7643	0.875	0.7	2990	32.0571	32	40
1	1	501.221	100.897	60	1.22476	0.65146	11.5626	0.873563	0.616571	3058	22.0571	32	40
1	1	501.221	100.897	60	1.22476	0.65146	11.5626	0.873563	0.616571	3058	19.1326	51	39
1	1	501.221	100.897	60	1.22476	0.65146	11.5626	0.873563	0.616571	3058	30.3328	32	37
1	1	501.221	100.897	60	1.22476	0.65146	11.5626	0.873563	0.616571	3058	31.0015	33	39
1	1	501.221	100.897	60	1.22476	0.65146	11.5626	0.873563	0.616571	3058	46.483	44	56
1	1	501.221	100.897	60	1.22476	0.65146	11.5626	0.873563	0.616571	3058	102.348	97	118
1	1	501.221	100.897	60	1.22476	0.65146	11.5626	0.873563	0.616571	3058	16.0889	30	123
1	1	501.221	100.897	60	1.22476	0.65146	11.5626	0.873563	0.616571	3058	38.3106	41	48

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1	1	21.2188	361.125	54	1.23161	0.634312	0.927003	0.874712	0.727273	3642	56.9063	57	68
2	2	24.0233	457.946	123	1.82866	0.827221	12.83156	0.921429	0.661538	3872	30.0135	30	37
3	3	35.6429	114.203	140	1.45781	0.72755	13.3512	0.908091	0.719167	4313	30.8071	31	38
4	4	39.8272	251.316	206	1.6179	0.79483	14.1953	0.918603	0.671632	5014	42.7573	45	51
5	5	39.5562	220.413	178	1.60005	0.731795	15.0345	0.927003	0.791111	5186	51.6679	52	56
6	6	39.3667	61.52	150	1.37573	0.327009	12.9198	0.90243	0.765306	6709	31.3923	33	38
7	7	39.3765	262.308	85	1.17568	0.527263	19.4021	0.894737	0.708233	3980	66.8235	49	57
8	8	45.323	24.1222	129	1.40448	0.702172	15.2034	0.956431	0.842424	4279	30.7442	32	35
9	9	57.3216	101.949	187	1.7115	0.825879	15.4304	0.912195	0.667657	6110	23.5819	24	28
10	10	55.5174	126.373	126	1.31697	0.450118	17.446	0.9	0.692309	1098	32.5238	33	40
11	11	65.4199	170.848	324	2.47752	0.914023	20.3108	0.78003	0.482143	16200	50	52	39
12	12	61.8618	292.139	135	1.65339	0.798019	15.133	0.819811	0.714146	3025	65.2921	48	53
13	13	62.3164	211.022	180	1.7728	0.875678	15.1368	0.904523	0.714286	8730	48.5	50	57
14	14	77.2222	315.178	45	1.82659	0.718165	7.5494	0.918367	0.714286	3366	71.8	71	58
15	15	77.3137	227.032	153	1.65509	0.730839	12.9373	0.921607	0.73	4477	29.2288	30	35
16	16	76.2075	362.452	52	1.31916	0.492494	8.21472	0.813703	0.734511	3314	66.3019	49	50
17	17	82.9479	94.3646	192	1.73679	0.421756	15.6353	0.923017	0.761905	8805	45.8596	47	54
18	18	96.0609	217.826	115	1.25503	0.604218	15.1003	0.912498	0.744753	4048	35.2	36	43
19	19	98.0968	190.519	137	1.34488	0.484908	15.8376	0.920561	0.724345	8240	65.8036	46	59
20	20	96.4796	467.951	102	1.72942	0.458226	11.4318	0.895632	0.72028	4108	39.8835	41	48
21	21	102.493	121.97	203	2.8942	0.938412	14.0769	0.853391	0.461314	8128	42.0412	42	53
22	22	100.551	38.6639	122	1.72624	0.815118	17.4324	0.917233	0.822449	3820	32.1311	33	39
23	23	101.027	98.6591	119	1.86847	0.846726	11.7736	0.925466	0.862222	8249	29.1879	30	35
24	24	104.427	203.069	120	1.76609	0.421208	17.8155	0.902278	0.714286	8111	32.8208	35	41
25	25	109.131	54.2347	199	1.31352	0.35046	18.9177	0.929907	0.743203	8907	44.7588	47	54
26	26	108.454	141.911	90	1.30509	0.423535	10.7087	0.918267	0.892308	3953	43.9222	45	53
27	27	110.122	243.843	115	1.22263	0.575319	12.1003	0.927639	0.746753	4134	35.9178	37	44
28	28	116.916	99.9877	91	1.22981	0.50217	10.1554	0.920455	0.818182	1016	49.9506	51	58
29	29	116.582	401.541	97	1.22722	0.51947	11.1704	0.899903	0.726224	3924	40.0708	41	48
30	30	122.37	281.45	200	1.59781	0.729569	15.9577	0.917431	0.714286	8226	46.16	47	53
31	31	128.98	121.02	93	1.28826	0.620308	11.2722	0.9	0.692308	4166	42.0409	42	53
32	32	127.437	447.737	102	1.71763	0.812068	11.4318	0.895632	0.866667	4048	39.466	41	48
33	33	133.750	196.913	277	1.43512	0.733144	10.70	0.923233	0.70844	9756	35.2202	37	46
34	34	136.911	428.439	101	1.33559	0.504271	11.3101	0.90893	0.765132	4128	40.8432	42	53
35	35	139.46	254.46	187	1.82919	0.837236	15.6304	0.916667	0.467857	9071	48.508	50	57
36	36	141.077	492	106	1.43370	0.72386	11.4174	0.929823	0.714239	4167	39.2112	40	51
37	37	146.702	221.56	84	1.39597	0.497144	10.2116	0.802224	0.777778	8132	48.9524	49	56
38	38	150	176.113	106	1.21719	0.570307	11.6174	0.898303	0.717259	8275	40.3302	42	50
39	39	161.276	449.232	103	1.32449	0.490811	11.5624	0.913013	0.848181	8157	38.3503	41	52
40	40	171.014	229.937	284	2.56596	0.91141	19.0158	0.890232	0.873233	13145	46.2852	48	56
41	41	165.611	211.881	101	1.34538	0.812253	11.3101	0.865963	0.708191	4301	42.3842	41	52
42	42	165.789	507.281	172	1.21828	0.571176	12.4334	0.810418	0.72518	4098	33.5902	35	47
43	43	169.352	42.2211	199	1.84139	0.835689	15.9177	0.921236	0.876071	8842	44.4322	46	54
44	44	182.642	191.45	349	1.96845	0.651332	21.0199	0.898872	0.606951	19671	56.3629	59	66
45	45	187.426	251.442	189	1.36125	0.480225	17.8159	0.902098	0.671975	4487	34.7829	35	42
46	46	187.183	410.238	109	1.74689	0.823039	11.7806	0.864778	0.599901	3878	35.1193	36	43
47	47	191.155	51.8	220	1.63125	0.750036	14.7166	0.923203	0.765714	8915	40.5227	41	51
48	48	192.106	120.497	142	1.33216	0.473836	13.4462	0.928105	0.728205	5924	41.7183	41	53
49	49	183.019	447.712	104	1.62036	0.807771	11.5973	0.896532	0.615205	2732	36.0769	36	44
50	50	184.271	346.037	187	2.02186	0.875021	15.4304	0.912195	0.436054	4596	24.5725	25	38
51	51	198.016	484.536	176	1.24109	0.403268	12.866	0.917368	0.801832	4126	32.746	33	40
52	52	207.241	176.43	46	1.54709	0.762022	7.63104	0.836364	0.431889	6768	126.261	142	166
53	53	208.225	323.431	160	2.41839	0.810505	14.2173	0.814266	0.634921	4698	28.1125	28	36
54	54	214.169	59.3133	118	1.79868	0.811208	17.7773	0.89697	0.653185	4887	31.6689	33	38
55	55	217.644	231.443	152	1.81213	0.833953	13.9116	0.926079	0.6	4545	29.9013	31	37
56	56	219.311	73.6682	118	1.62943	0.78953	13.7773	0.912475	0.791444	4274	42.3819	41	53
57	57	222.911	99.6111	144	1.47822	0.736453	13.5106	0.917197	0.705082	4120	30.8946	32	37
58	58	222.402	489.059	102	1.41952	0.723819	11.3961	0.894737	0.462338	3850	37.7451	38	47
59	59	227.269	450.393	84	1.22251	0.51323	10.2118	0.923233	0.765136	3723	44.2214	46	52
60	60	232.114	200.992	123	1.40951	0.704741	12.5143	0.924612	0.745455	4194	34.0976	35	42
61	61	235.639	393.375	166	2.24611	0.904532	14.5381	0.903394	0.81484	8447	26.7492	27	30
62	62	236.501	159.211	209	1.50163	0.747971	15.3128	0.93722	0.765566	8114	42.6073	46	56
63	63	236.101	297.94	134	1.48161	0.710232	17.0419	0.911563	0.761164	4302	32.1045	33	39
64	64	237.048	27.3982	104	1.59005	0.777477	11.9073	0.923201	0.493333	3989	38.3558	40	47
65	65	242.184	51.7053	207	1.81456	0.83441	18.2343	0.927895	0.723776	7026	33.8903	33	43
66	66	243.917	439.297	109	1.58813	0.716882	14.7265	0.921034	0.6	4021	37.2315	38	45
67	67	252.39	397.256	172	1.71191	0.811632	16.7788	0.900526	0.46617	4853	27.0323	28	33
68	68	265.139	261.346	296	1.5517	0.77697	19.3477	0.86024	0.465158	9550	32.483	34	40
69	69	267.379	38.9321	254	1.38602	0.492434	17.9834	0.852348	0.711485	8097	35.815	35	43
70	70	274.18	118.426	278	1.31653	0.463466	18.4138	0.903337	0.861903	16009	37.5843	40	47
71	71	274.102	11.082	122	1.34993	0.471789	12.4634	0.920242	0.72519	4195	34.418	36	41
72	72	285.584	491.87	298	1.62601	0.716536	19.4788	0.814208	0.80097	9878	31.7987	33	39
73	73	267.241	474.126	278	2.44337	0.912713	18.8138	0.810496	0.620526	9378	33.3741	34	40

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1	9	283.616	101.611	127	1.6886	0.405787	17.7162	0.913669	0.661438	4629	36.4173	37	29	44
1	9	284.665	101.671	173	1.33834	0.759964	14.9113	0.925134	0.759772	1670	44.3333	46	35	54
1	9	281.69	289.917	84	1.53434	0.759511	10.3418	0.903226	0.661154	4211	50.131	51	39	62
1	9	286.312	80.4481	154	1.13337	0.470416	16.0028	0.916667	0.733333	4526	29.3896	30	23	26
1	9	292.389	105.311	90	1.08188	0.393714	10.7017	0.909091	0.713802	4131	43.3233	46	36	56
1	9	292.389	105.311	90	1.08188	0.393714	10.7017	0.909091	0.713802	4131	43.3233	46	36	56
1	9	292.3	312.755	106	1.33544	0.759539	11.472	0.930533	0.64327	4700	38.0093	39	29	44
1	9	300.374	67.9346	107	1.27271	0.759839	11.4714	0.930533	0.64327	4700	64.3231	46	36	54
1	9	302.3	312.755	106	1.33544	0.759539	11.472	0.930533	0.64327	4700	64.3231	46	36	54
1	9	310.561	318.784	149	2.13765	0.908053	13.7273	0.840909	0.928571	8947	60.4331	60	41	74
1	9	305.199	314.341	47	1.29753	0.837203	7.48182	0.846134	0.6875	3760	85.4345	88	63	106
1	9	313.183	388.246	224	2.05955	0.874094	16.818	0.910295	0.59891	9248	43.7748	43	32	50
1	9	312.337	242.253	359	2.13391	0.883638	21.3397	0.810381	0.569141	15817	55.2006	58	42	89
1	9	315.082	322.148	182	1.40117	0.733934	13.2227	0.928571	0.738842	9174	52.0519	55	41	64
1	9	322.85	31.4724	149	1.27189	0.835902	16.4082	0.910615	0.93216	4364	26.773	38	20	32
1	9	327.5	356.405	138	1.24078	0.824816	18.1935	0.923977	0.752181	4388	31.5196	33	25	38
1	9	317.5	322.588	241	1.64339	0.792838	17.3172	0.893211	0.914211	8916	41.1152	42	21	51
1	9	312.971	48.3441	151	1.32118	0.611977	13.8636	0.920732	0.723562	4219	28.1391	29	22	31
1	9	316.143	328.607	26	1.12488	0.460666	5.97082	0.875	0.664667	5659	202.107	215	166	218
1	9	332.064	282.676	173	1.60916	0.792613	14.8415	0.933135	0.758772	4693	27.1618	28	21	31
1	9	338.062	356.634	145	1.37417	0.695891	13.5875	0.923487	0.71339	8423	38.0497	40	47	69
1	9	338.17	31.9816	217	2.18678	0.899318	16.4221	0.90193	0.615873	4393	21.1751	21	17	24
1	9	338.408	69.4079	74	1.23377	0.516432	9.82698	0.98118	0.690909	7203	94.0026	99	76	114
1	9	338.361	137.435	11	1.13876	0.478393	3.71241	0.783711	0.8873	210	19.0809	19	14	22
1	9	312.176	312.176	148	1.2356	0.839107	13.7273	0.86937	0.621188	4306	29.0946	30	22	33
1	9	370.203	202.324	186	2.20917	0.89501	16.5381	0.92272	0.65872	4386	27.6467	29	21	34
1	9	377.717	170.037	404	1.31494	0.630488	22.6801	0.920876	0.708294	20716	31.2722	32	38	64
1	9	344.188	243.899	228	1.4413	0.734718	20.4338	0.931818	0.788142	12032	34.6324	37	27	46
1	9	375.638	68.116	69	1.32144	0.73403	9.37302	0.881104	0.427273	3364	51.3423	52	41	62
1	9	393.181	691.879	155	1.6491	0.800655	16.4882	0.922619	0.615823	5724	36.3119	38	29	45
1	9	392.202	291.197	213	1.22925	0.632608	16.4882	0.926007	0.739583	4337	29.7512	30	22	38
1	9	391.713	407.231	195	1.80111	0.831707	15.737	0.921171	0.714286	9007	46.1893	48	35	58
1	9	404.037	455.266	82	1.57912	0.773977	10.2179	0.911111	0.759259	3345	48.1098	51	38	59
1	9	418.117	178.087	60	1.24155	0.595239	6.74039	0.645645	0.646647	4988	81.3	83	63	101
1	9	430.121	389.29	124	1.23756	0.599125	12.5871	0.903109	0.738055	8216	31	35	34	41
1	9	434.472	217.336	250	1.45526	0.72659	17.8412	0.91985	0.730391	9416	37.464	39	30	46
1	9	430.222	486.361	72	1.21882	0.587787	9.37481	0.910049	0.72	3710	51.9441	52	41	81
1	9	438.336	245.49	329	1.93118	0.957156	20.2195	0.813602	0.419181	11118	26.1243	36	27	42
1	9	444.312	381.171	316	2.27398	0.888116	20.0393	0.843388	0.748111	13338	54.119	58	43	71
1	9	441.239	288.096	230	1.75024	0.820708	17.1127	0.927418	0.484324	9481	41.1348	42	31	51
1	9	442.21	37.6443	143	1.74234	0.607001	13.4933	0.923981	0.747392	9280	57.7622	80	45	70
1	9	441.895	437.769	52	1.60181	0.700786	6.13656	0.95552	0.727357	3333	67.9123	70	53	81
1	9	450.907	339.883	181	2.23932	0.493718	14.3173	0.92	0.731916	4391	27.2733	27	20	35
1	9	452.118	424.193	119	1.29823	0.637708	12.3032	0.901515	0.708333	4146	31.6102	36	27	42
1	9	452.477	462.156	128	1.13795	0.502405	12.7682	0.920863	0.757396	4253	33.3381	35	26	40
1	9	455.773	440.5	44	1.23815	0.50786	7.48482	0.88	0.785714	3288	35.4364	77	62	90
1	9	476.277	407.551	130	1.61995	0.705982	12.8435	0.915493	0.714286	7021	54.6071	56	44	66
1	9	479.27	187.426	152	1.48658	0.740718	12.5116	0.853933	0.433233	4931	32.9728	33	26	40
1	9	479.137	213.248	121	1.23349	0.49319	12.4122	0.903889	0.715926	4303	35.9928	37	28	43
1	9	480.888	10.1837	49	1.4708	0.722302	7.89845	0.901407	0.777378	3891	120.224	122	92	147
1	9	485.018	132.565	188	1.6403	0.792634	14.4233	0.933333	0.777778	4290	25.2357	26	20	32
1	9	486.875	230.544	160	1.16375	0.48109	14.773	0.946746	0.784314	8061	90.3063	93	41	62
1	9	489.327	41.2515	183	1.17225	0.73133	16.1983	0.911602	0.705128	4206	25.4909	27	20	31
1	9	488.927	211.45	191	1.19913	0.697839	15.5883	0.927186	0.703106	9350	48.9329	50	38	60
1	9	493.91	94.3349	212	2.14811	0.895036	16.4234	0.921738	0.458183	7500	38.3774	38	28	41
1	9	495.135	229.708	192	1.72749	0.637671	15.6333	0.934039	0.761505	8831	45.9948	48	36	55
1	9	497.018	268.188	178	2.12112	0.881898	13.0385	0.92228	0.706319	6413	36.0181	38	27	44

EV Table 2.doc

Example of the summary output of AnalysedNA.m program
(summary for 10 3 by 3 montage images)

1	1107	163.912	79.3918	1.58889	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
2	22.0103	14.334	50.5906	22.6366	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
3	1305	169.036	86.8722	1.60311	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
4	33.0245	13.3362	51.2608	21.2307	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
5	1399	166.57	80.0679	1.58728	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
6	31.0313	16.2062	54.4417	25.1763	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
7	1380	172.073	88.7143	1.60109	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
8	33.7368	15.9902	52.503	24.193	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
9	1418	171.921	90.64	1.58887	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
10	34.7315	16.8358	53.6367	26.3398	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
11	1410	165.142	84.6806	1.60542	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
12	34.3446	15.2864	53.9055	23.8472	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
13	1756	129.864	98.7039	1.57806	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
14	28.8901	17.0123	63.0689	23.6082	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
15	1280	171.387	84.1239	1.59201	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
16	32.0602	13.7194	51.3961	20.836	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
17	1270	166.53	86.5091	1.60367	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
18	31.3669	13.0149	50.6976	21.2407	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
19	1425	159.606	82.610	1.57886	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393
20	34.4382	16.3199	53.7958	21.6086	0.388733	0.724661	0.137956	14.0612	3.316	0.905327	0.0350365	0.701210	0.075176	6149.26	3166.95	11.539	18.352	62.9464	19.9393

CLAIMS

What is claimed is:

1. A method of predicting a property of a manipulation of cells based
5 upon a descriptor, said method comprising:
 providing a plurality of cells;
 manipulating said plurality of cells;
 capturing a morphological value from said plurality of cells;
 assigning a degree of presence of said morphological value; and
10 storing said morphological value and said degree of presence;
 wherein said descriptor is derived from a first component of a cell and
a second component of said cell, said capturing said morphometric value from said
plurality of cells comprises determining a relationship between said first component
and said second component.
- 15 2. The method of claim 1 wherein said first component and said second
component are selected from a protein, a protein modification, a nucleic acid, a lipid,
a carbohydrate, a subcellular structure and an organelle.
3. The method of 1 wherein said step of manipulation occurs in a manner
selected from a electrical source, a chemical source, a thermal source, a gravitational
20 source, a nuclear source, a temporal source, and a biological source
4. The method of claim 3 wherein said chemical source is selected from a
pharmacological candidate and a drug screening library.
5. The method of claim 1 wherein said morphological value is selected
from a count, an area, a perimeter, a length, a breadth, a fiber length, a fiber breadth, a
25 shape factor, a elliptical form factor, an inner radius, an outer radius, a mean radius,
an equivalent radius, an equivalent sphere volume, an equivalent prolate volume, an
equivalent oblate volume, an equivalent sphere surface area, an average gray value, a
total gray value, and an optical density.
6. The method of claim 1 wherein said degree of presence is
30 multiple of a quantized value.

7. A computer program product for populating a database with manipulated biological information, said computer program product comprising:
- code for providing a plurality of cells in various stages of the cell cycle, said stages of the cell cycle including at least one selected from interphase, G0 phase, G1 phase, S phase, G2 phase, M phase, prophase, prometaphase, metaphase, anaphase, and telophase;
 - code for manipulating said cells in said various stages of cell cycle development to form a plurality of manipulated cells;
 - code for capturing an image of said plurality of manipulated cells;
 - code for determining a descriptor from said image for said manipulated cells;
 - code for populating a database with said descriptor;
 - wherein said image includes a first component of a cell and a second component of said cell; and
 - a computer readable storage medium for holding the codes.
8. The computer program product of claim 7 wherein said first component and said second component are selected from a protein, a protein modification, a nucleic acid, a lipid, a carbohydrate, a sub-cellular structure and an organelle.
9. The computer program product of claim 7 wherein said image is a digitized representation of said plurality of manipulated cells.
11. The computer program product of claim 9 wherein said digitized representation provides a density value of said plurality of manipulated cells.
11. The computer program product of claim 7 wherein said descriptors comprise numeric or logical values.
12. The computer program product of claim 11 wherein said values further comprises a nucleotide.
13. The computer program product of claim 11 wherein said values further comprises an amino acid letter.
14. A system for capturing images of cells or cell structures, the system comprising:
- a cell holder comprising a plurality of sites in a spatial orientation, each of the sites being capable of holding a plurality of cells to be imaged;

an image capturing device coupled to the cell holder, the image capture device being adapted to capture at least one image in at least one of the plurality of sites;

5 an illumination apparatus comprising a liquid light guide coupled to the plate for highlighting the plurality of cells in a relatively even spatial manner for image capturing purposes;

an image processing device coupled to the image capturing device, the image capturing device being adapted to convert the image into a digital representation; and

10 a database storage device comprising a database management element coupled to the image capturing device, the database storage device being adapted to retrieve the digital representation of the image from the image processing device and storing the digital representation.

15 15. The system of claim 14 further comprising a stage comprising a device for moving the cell holder in a spatial direction to traverse across the cell holder in the spatial orientation.

20 16. The system of claim 14 wherein the illumination apparatus comprises sub-elements, at least one of the sub-elements being positioned away from the image capturing device to prevent a possibility of vibration from the one sub-elements to be transmitted to the image capturing device.

17. The system of claim 14 wherein the digital representation comprises a plurality of regions and objects.

18. The system of claim 14 further comprising a computing device connected between the database storage device and the image processing device.

25 19. The system of claim 14 wherein the image capturing device comprises a magnification of at least 1X and greater to capture the image of the site.

20. The system of claim 14 wherein the plurality of sites comprises at least 96 sites.

30 21. The system of claim 14 wherein the liquid light guide characterized as a flexible member that substantially prevents vibration from the an element of the illumination apparatus to be transferred to the image capturing device.

22. The system of claim 14 wherein the spatial direction can be selected from an x-direction, a y-direction, or a z-direction in a Cartesian coordinate system.

23. The system of claim 14 wherein the each of the sites comprises
5 a volume that is sufficient to prevent a solution therein from evaporating in a substantial manner that may influence the image capturing.

24. A method for identifying a mechanism of action for a first compound, the method comprising the steps of:
receiving the first compound;
10 measuring at least one feature of a cellular phenotype to define a target phenotype;
identifying additional compounds providing a feature similar to the feature identified in the measuring step; and
characterizing the first compound in terms of distance from a specific
15 target phenotype having known characteristics.

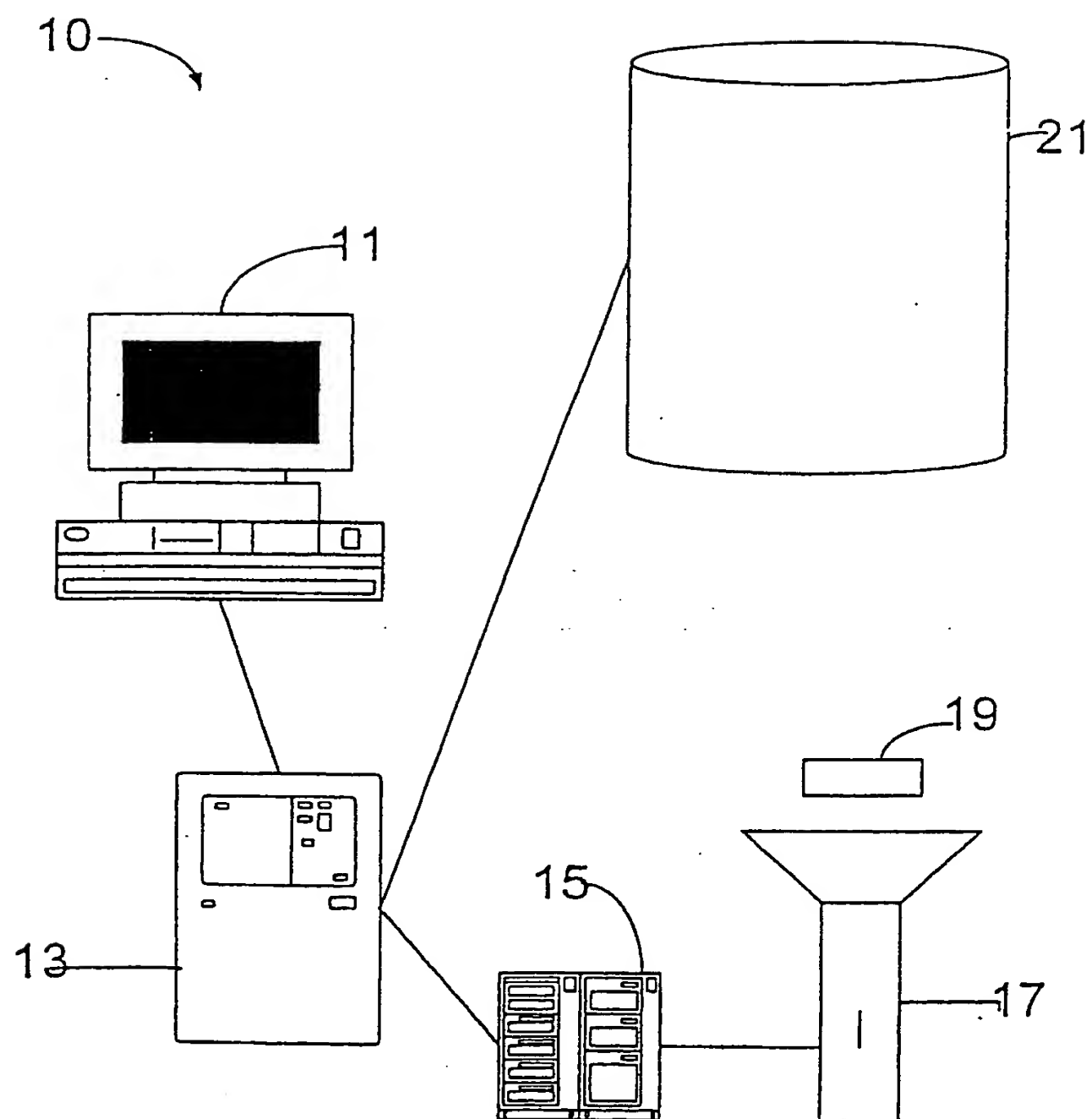
25. The method of claim 24 comprising the further step of storing the additional compounds and their associated phenotypes in a database.

26. A method for identifying a mechanism of action for a cellular stimulus, the method comprising the steps of:
20 receiving cells of interest;
measuring at least one feature of the cells to define and quantify a target phenotype;
identifying additional compounds providing a feature similar to the feature identified in the measuring step; and
25 characterizing the first compound in terms of distance from a specific target phenotype having known characteristics.

27. A method for identifying information relevant to at least one of a mechanism of action and cellular activity by utilizing assay data to elucidate a phenotype, the method comprising the steps of:

30 identifying a target protein;
identifying positive and negative biochemical hits related to the target protein;
defining the target phenotype utilizing the positive and negative hits;

identifying other compounds providing similar features; and
characterizing the first compound in terms of distance from a specific
target phenotype having known characteristics.

**FIG. 1**

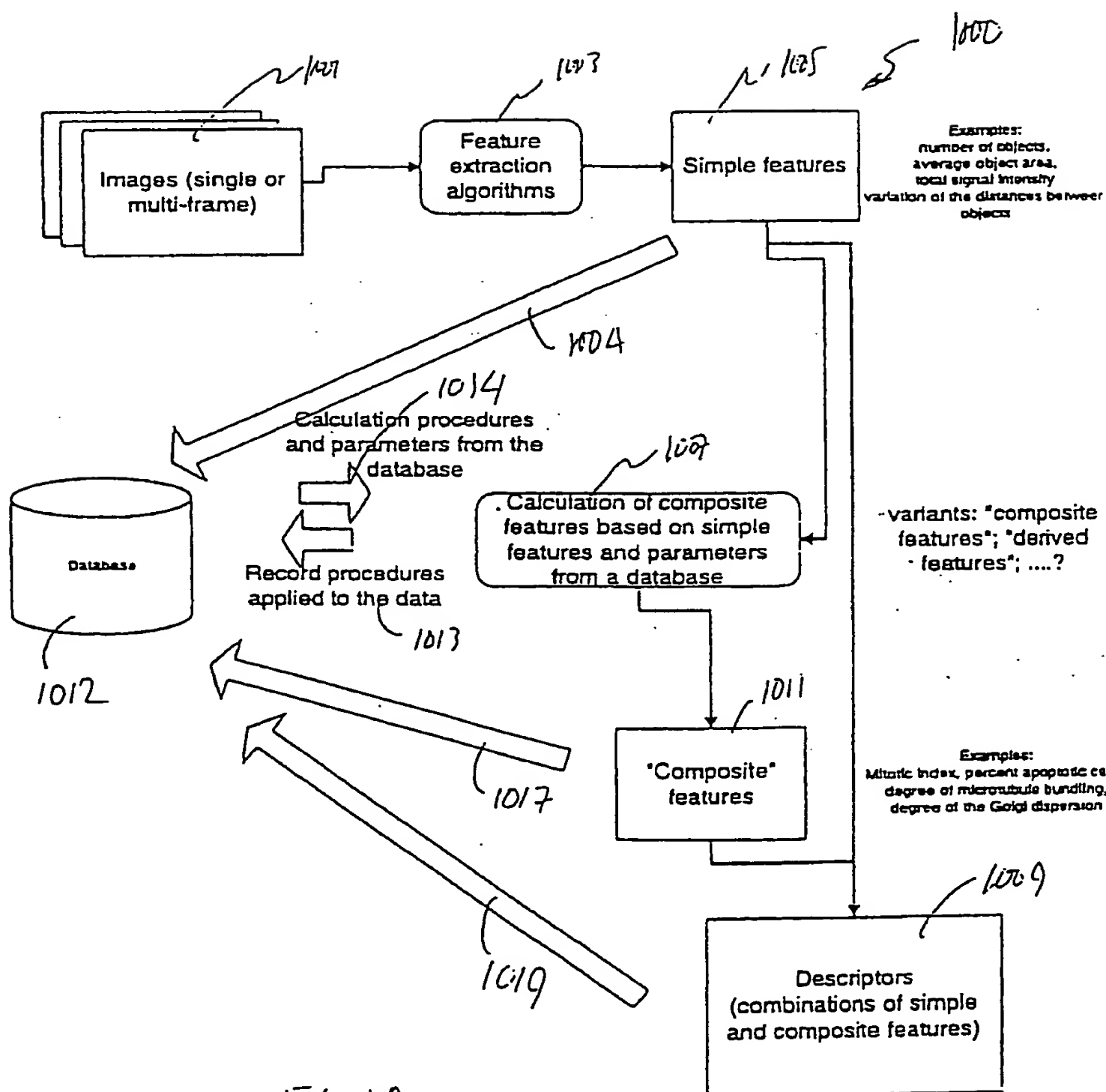


FIG. 1A

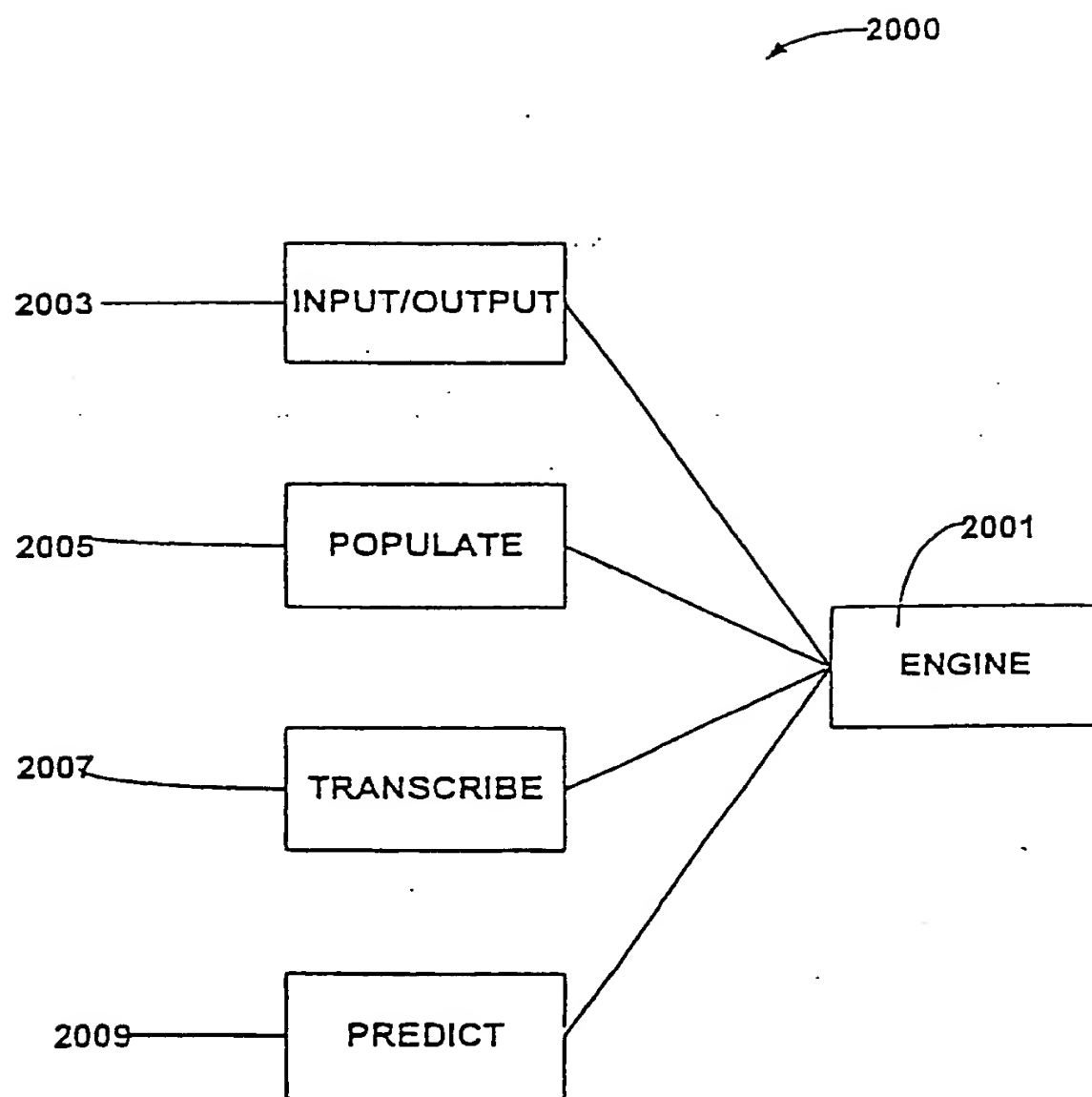


FIG. 1B

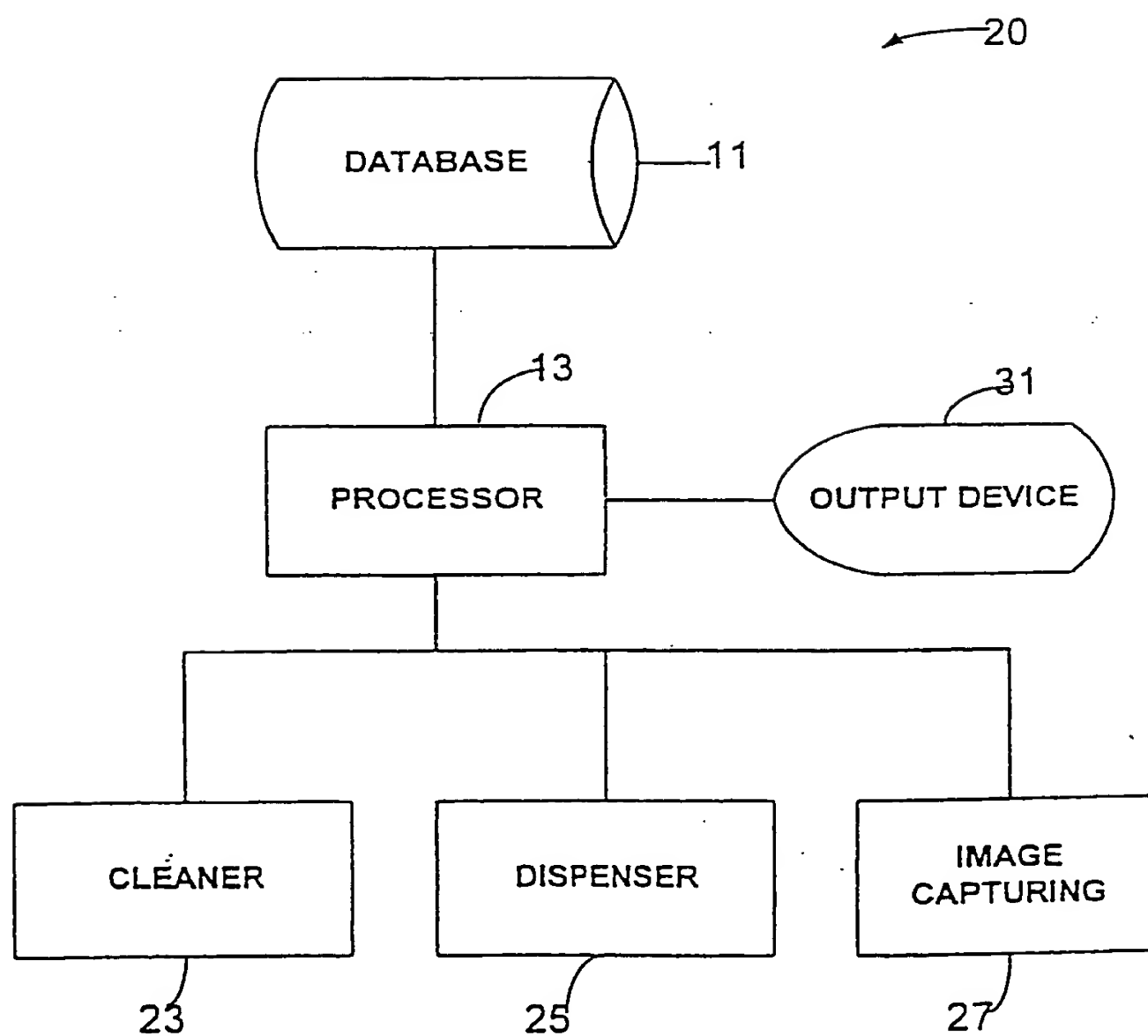


FIG. 2

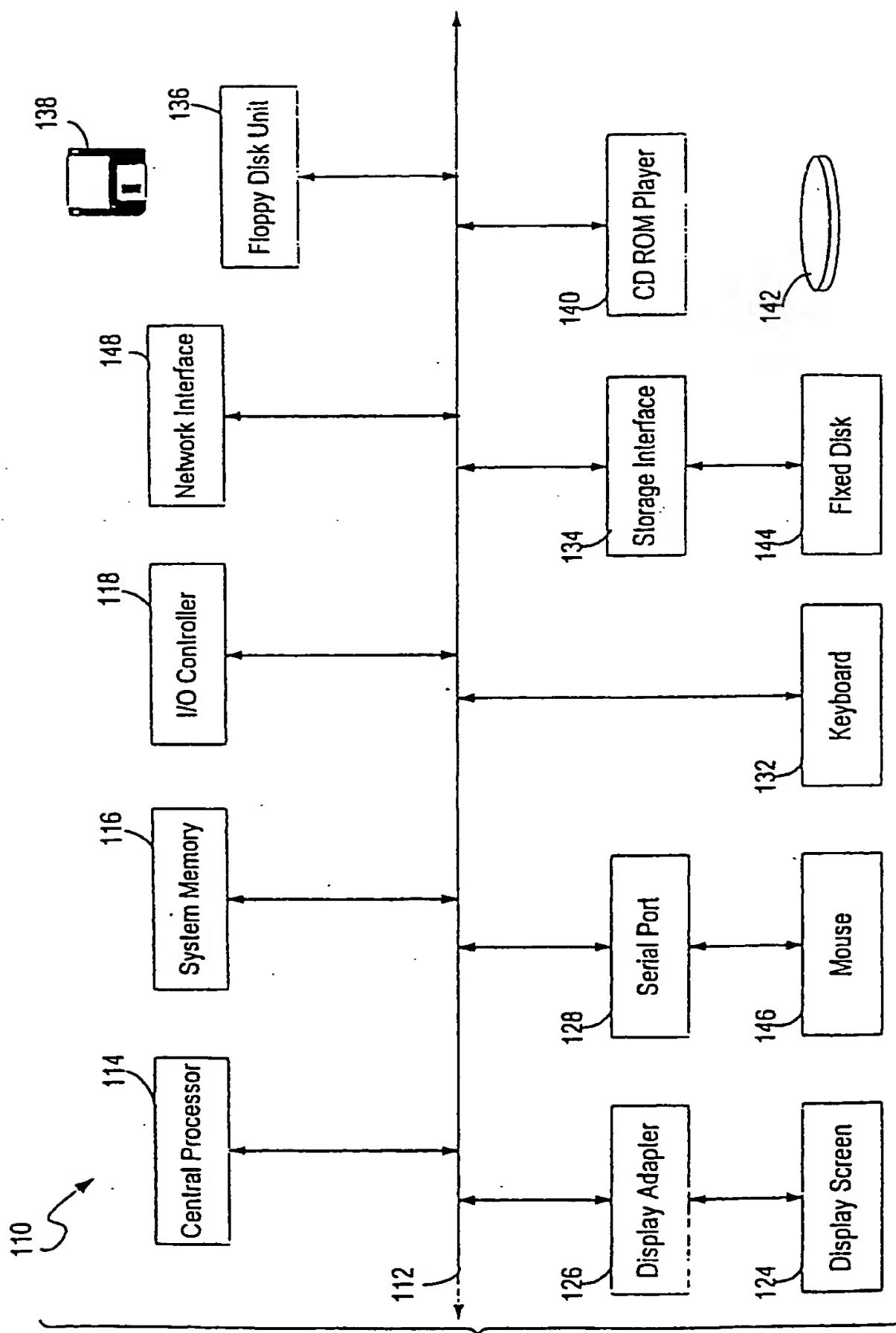


Fig 3

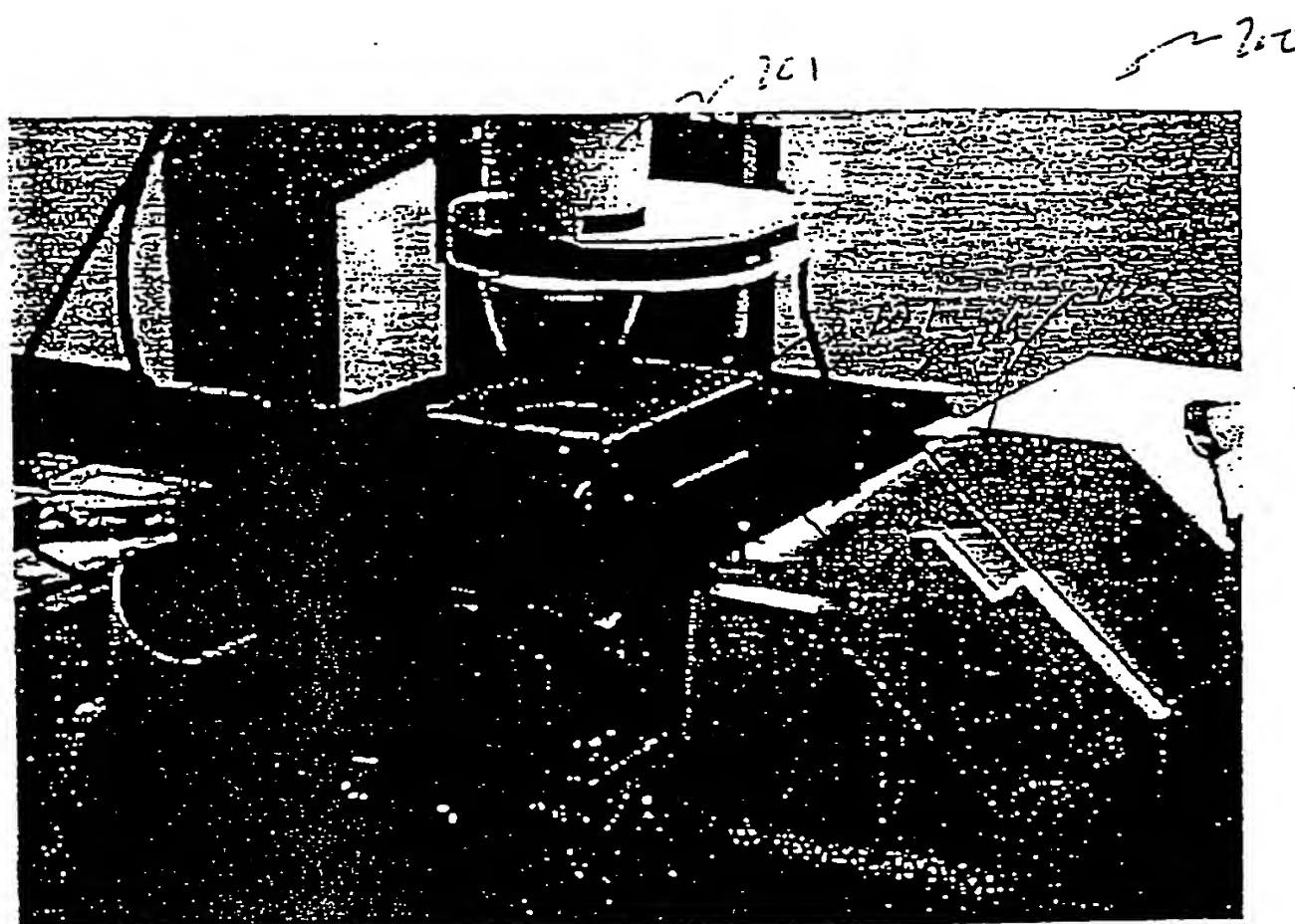


FIG. 4

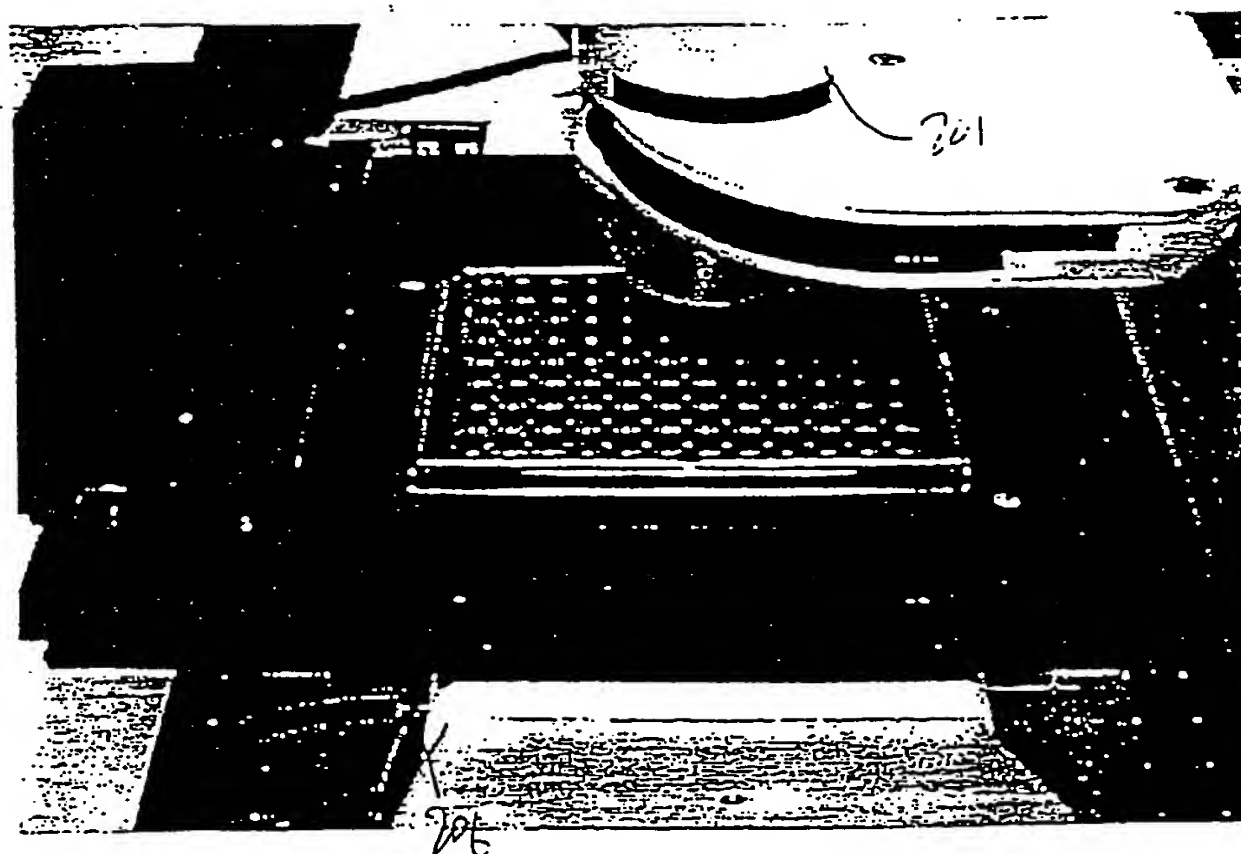
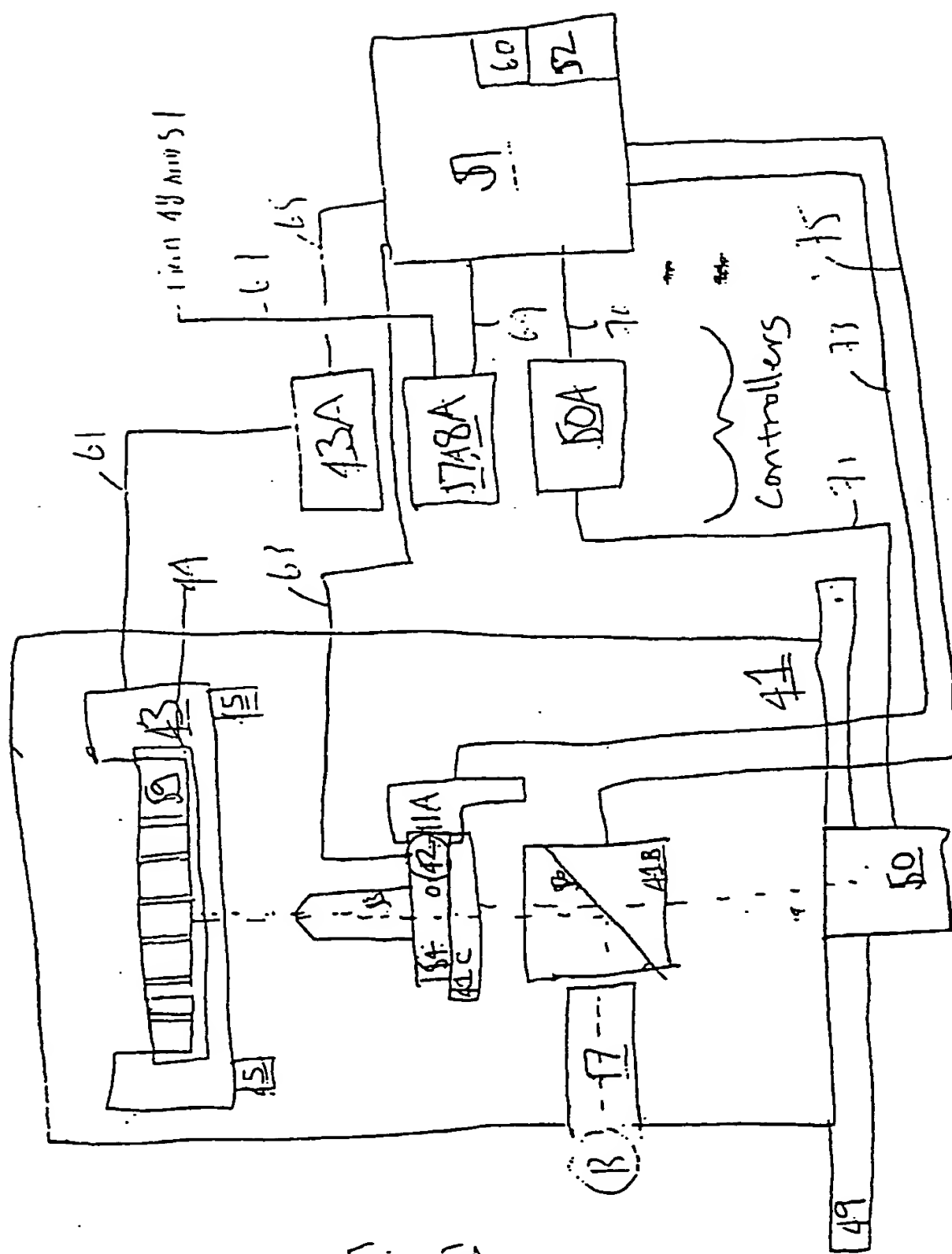


FIG 5



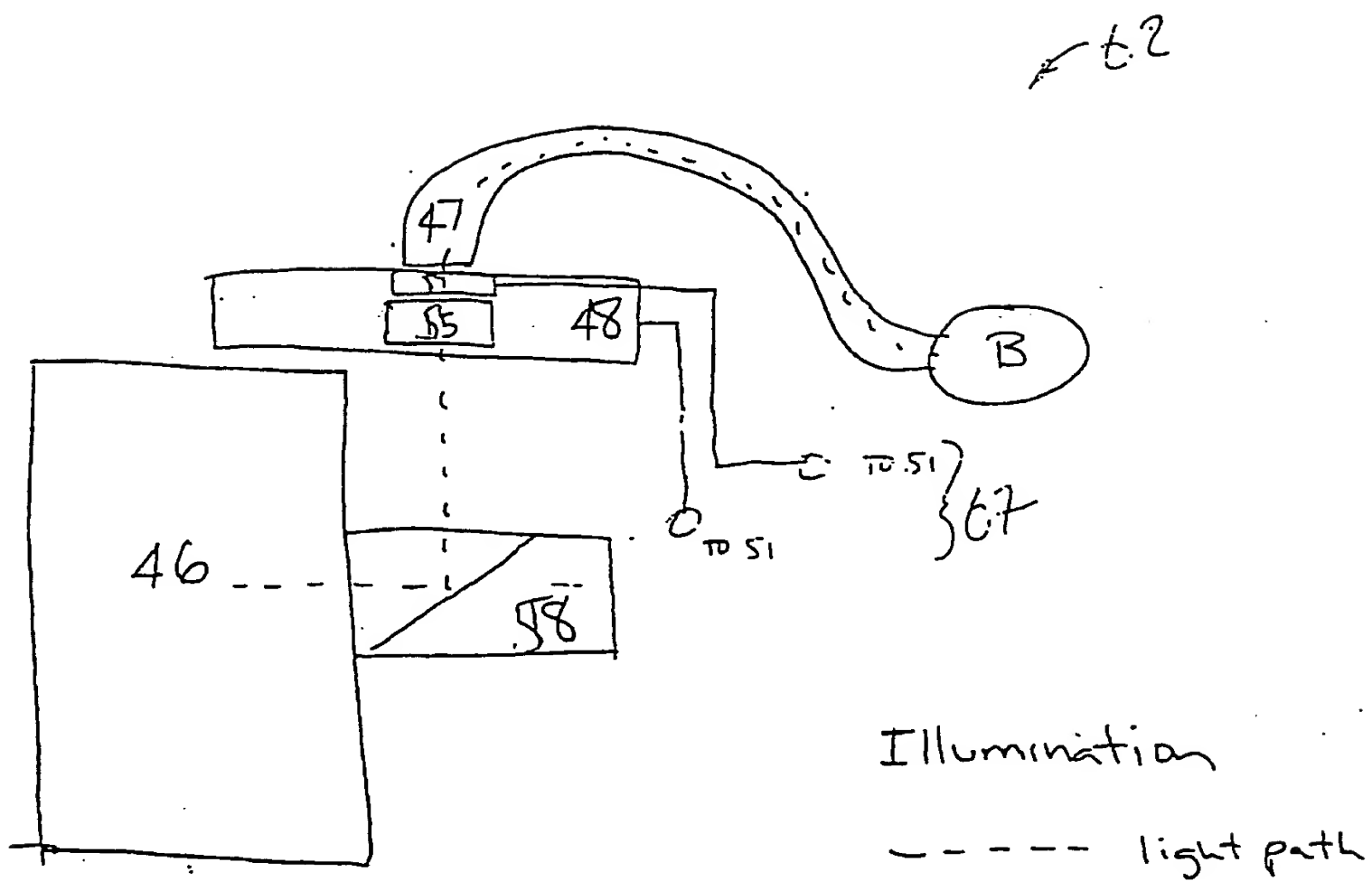


FIG. 5B

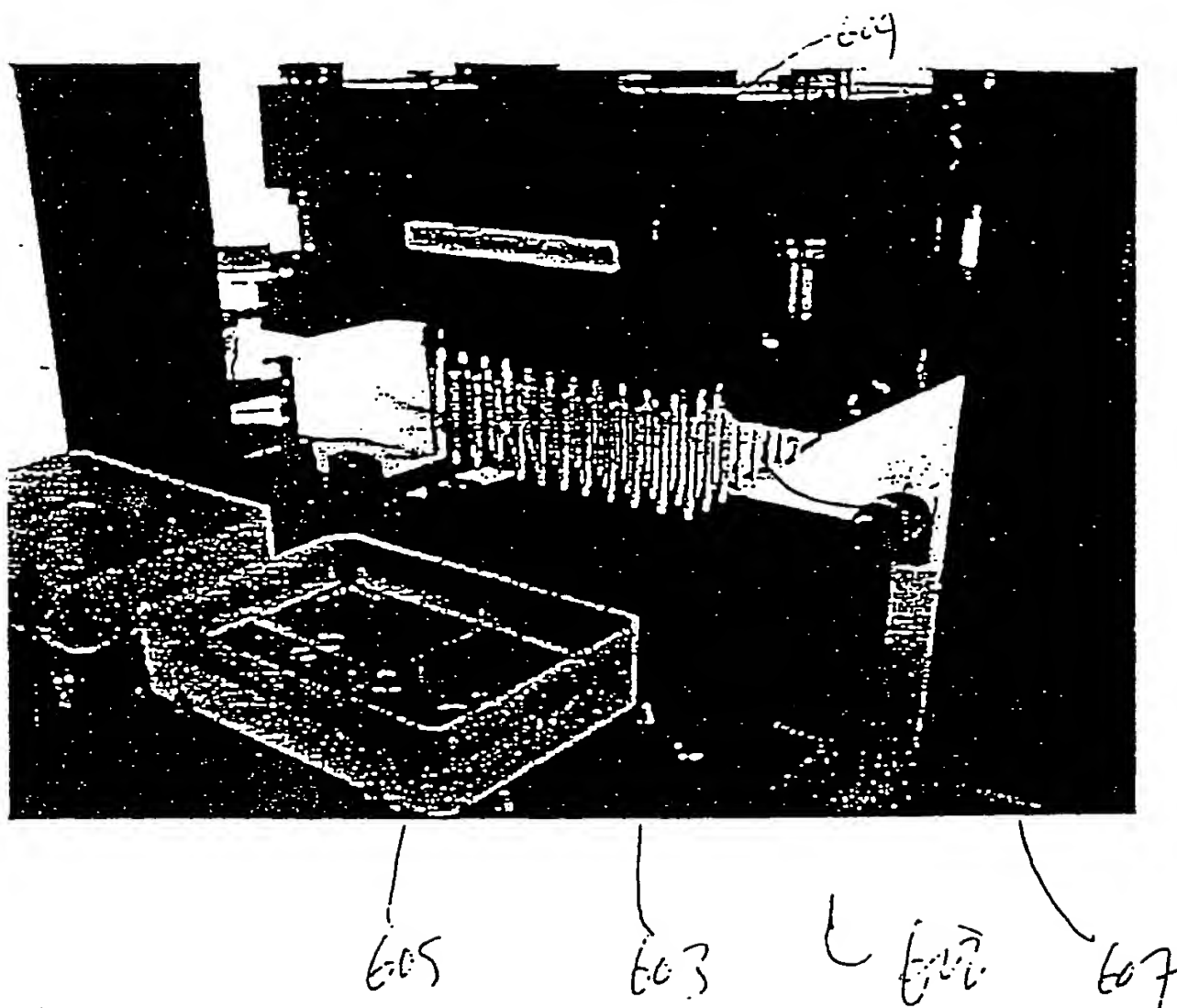


FIG. 6

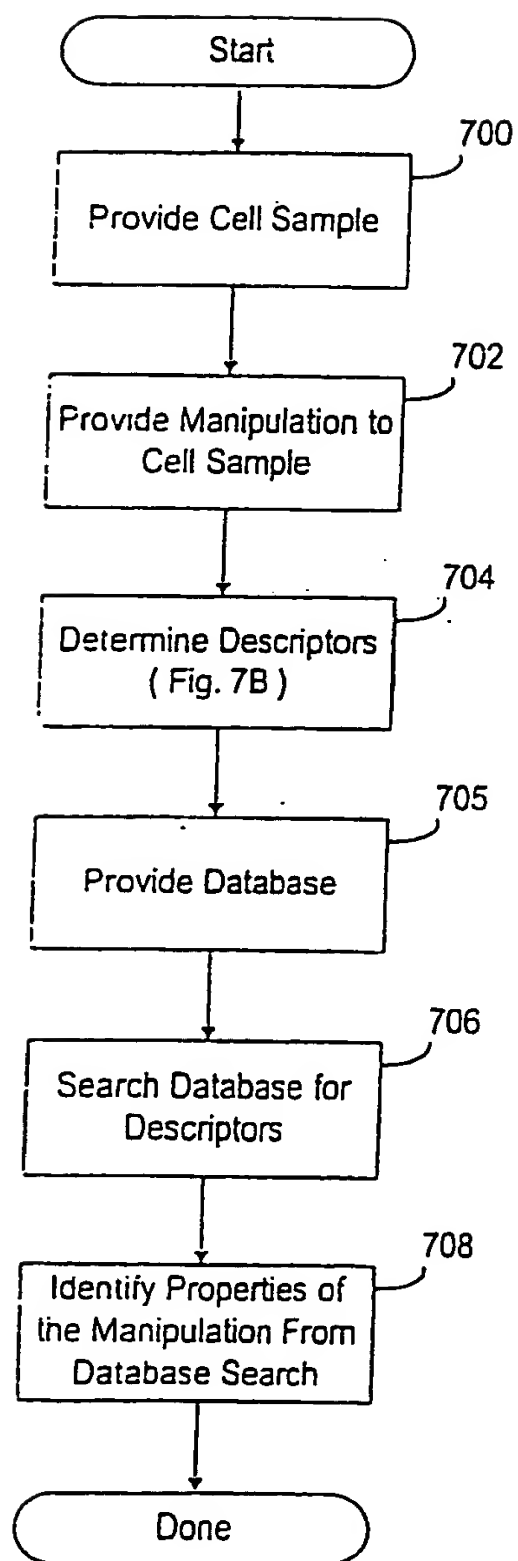


Fig. 7A

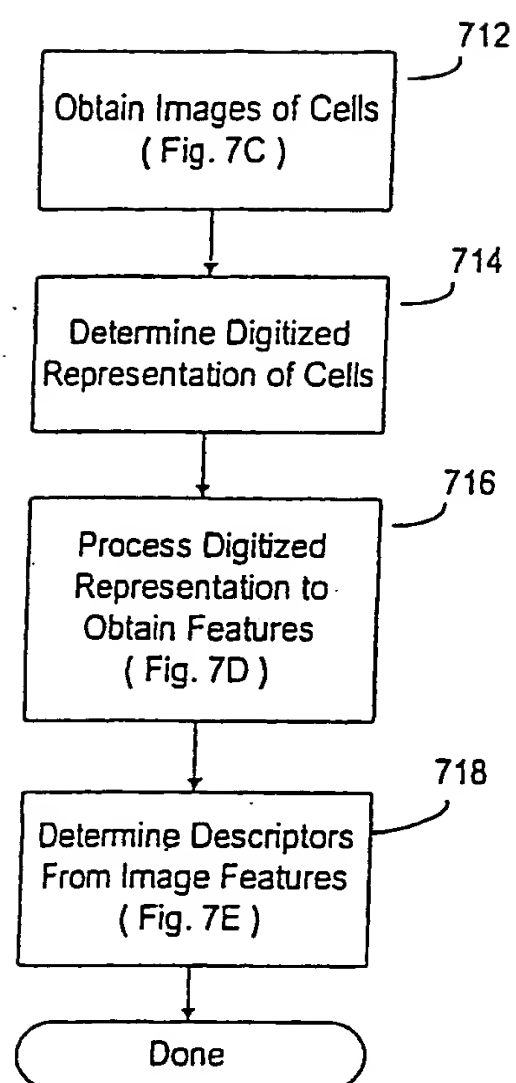


Fig. 7B
Step 704 of Fig. 7A

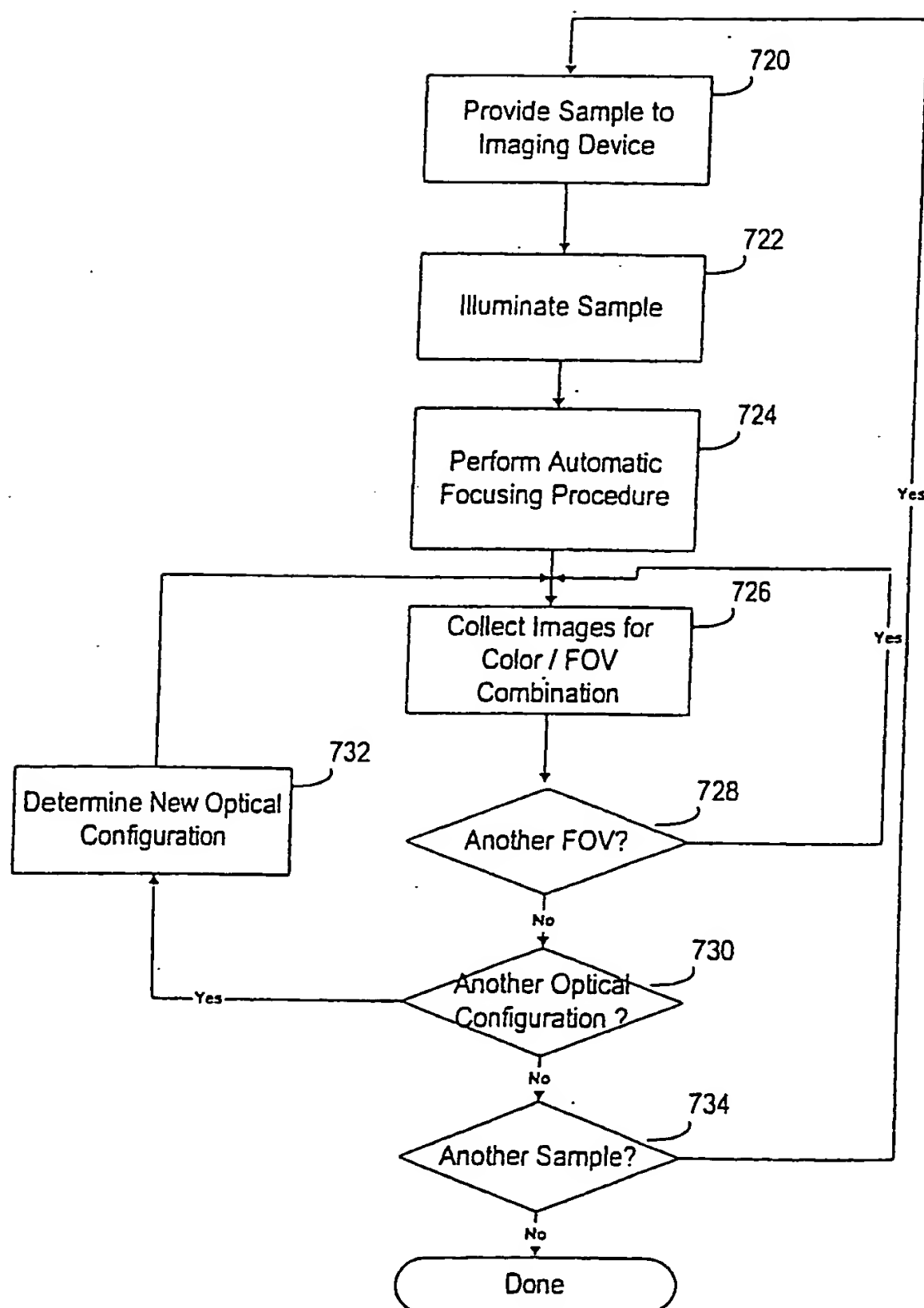


Fig. 7C
Step 714 of Fig. 7B

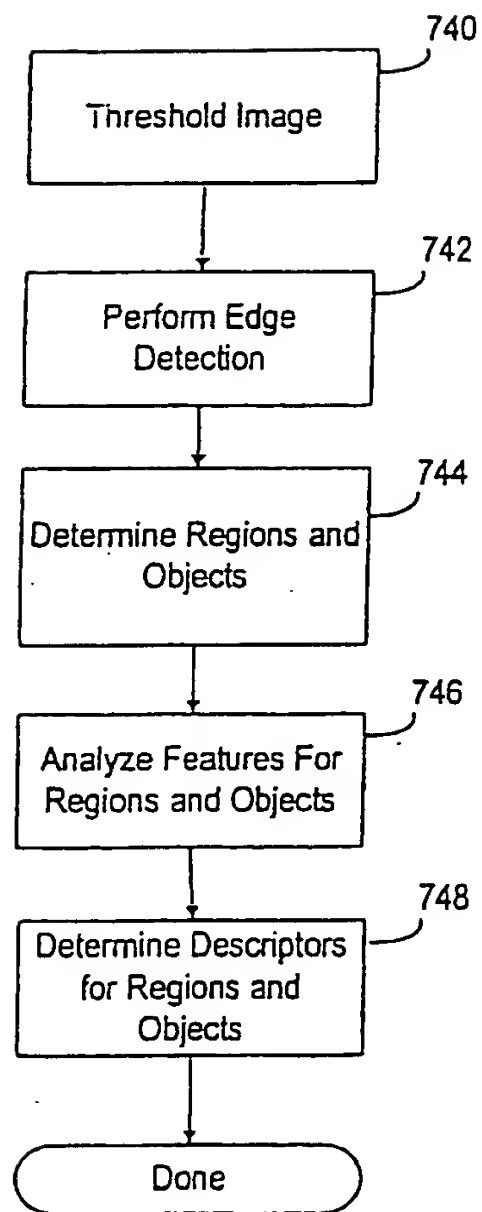


Fig. 7D
Step 716 of Fig. 7B

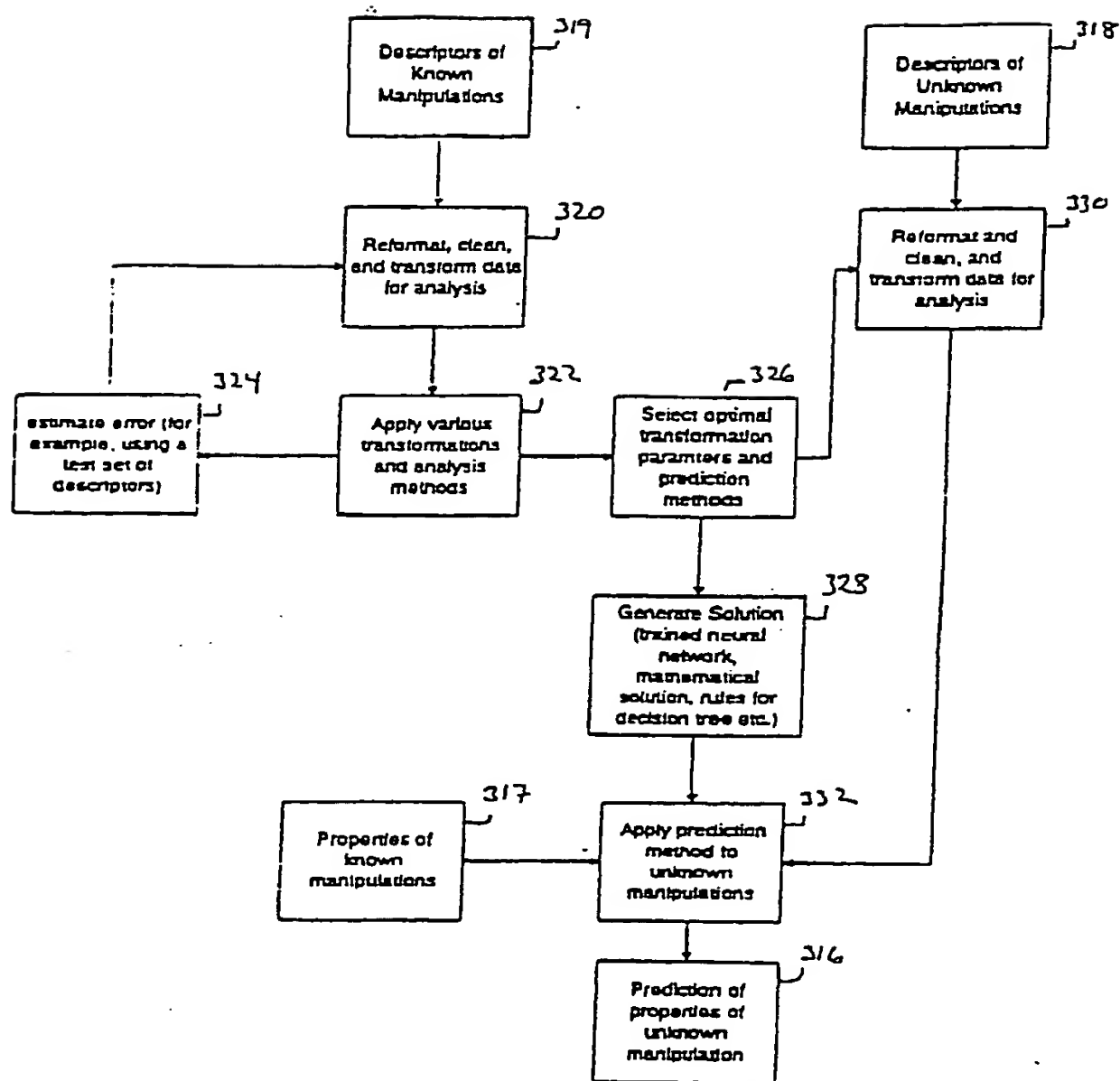


FIG. 7E

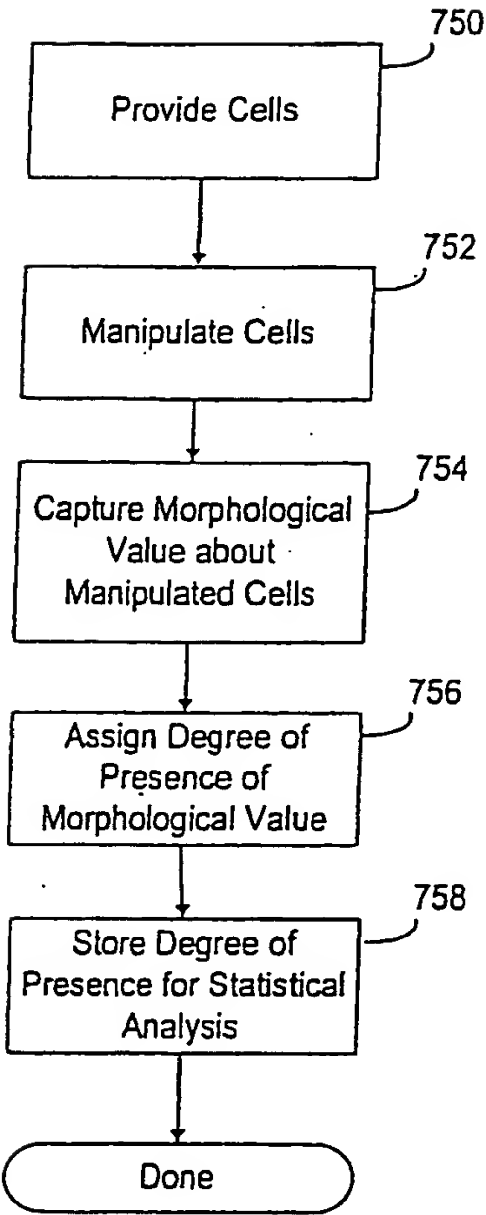


Fig. 7F

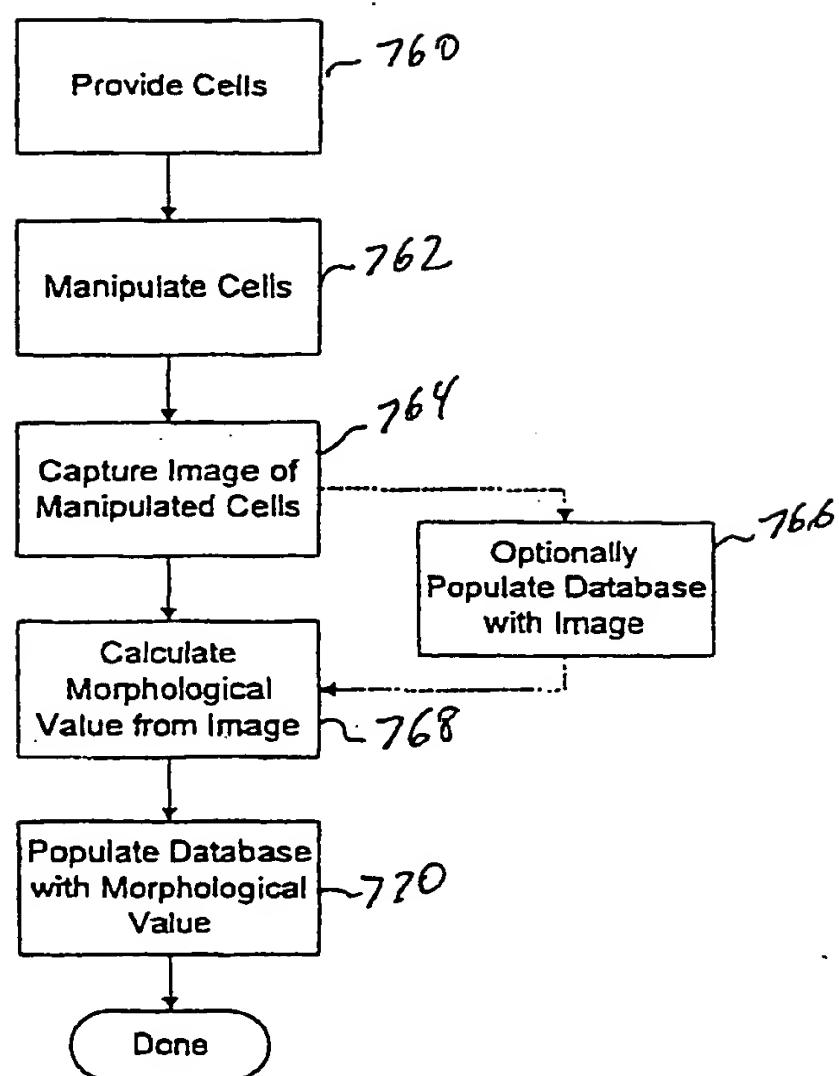


Fig 7G

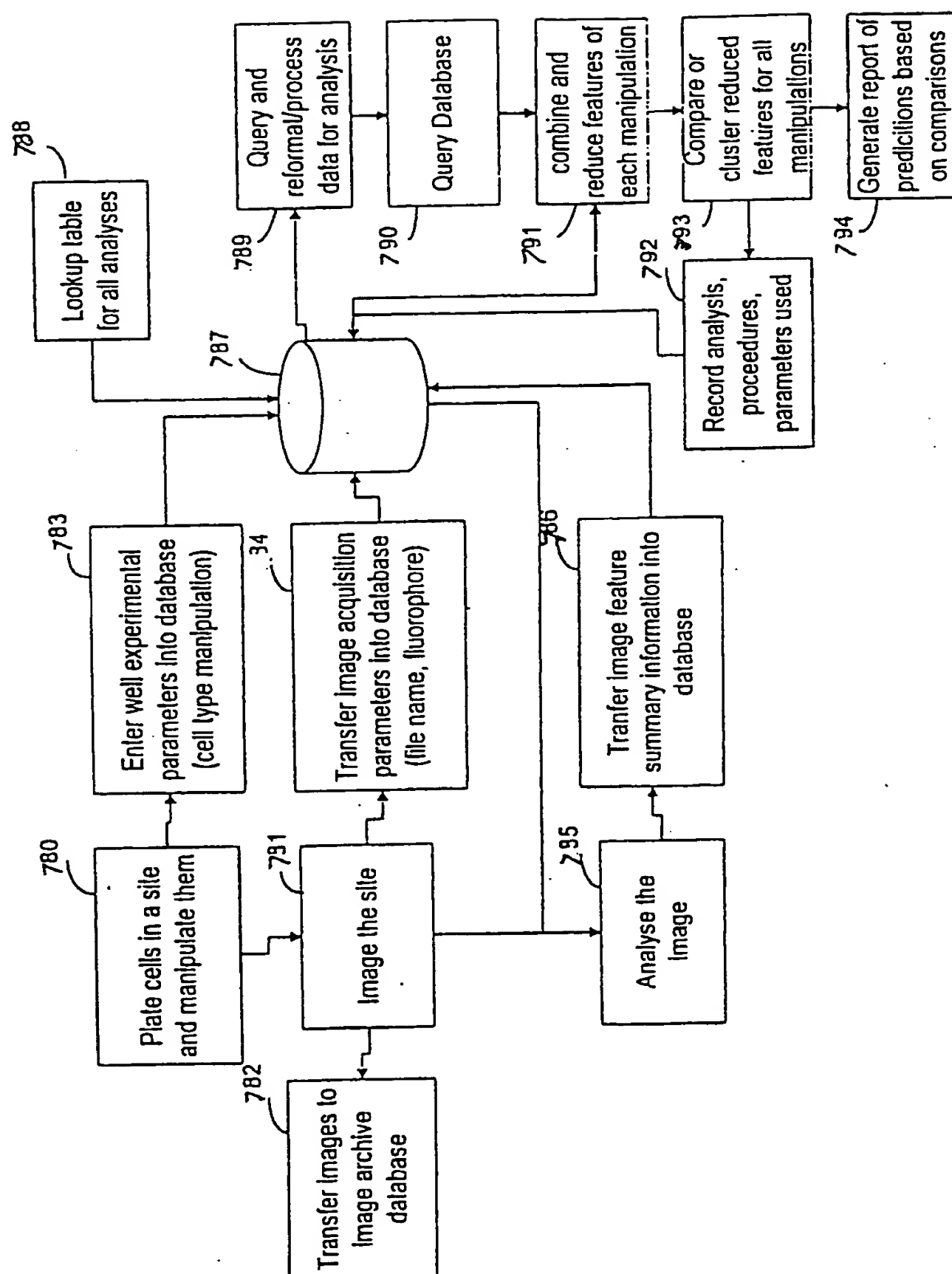


Fig. 7 H

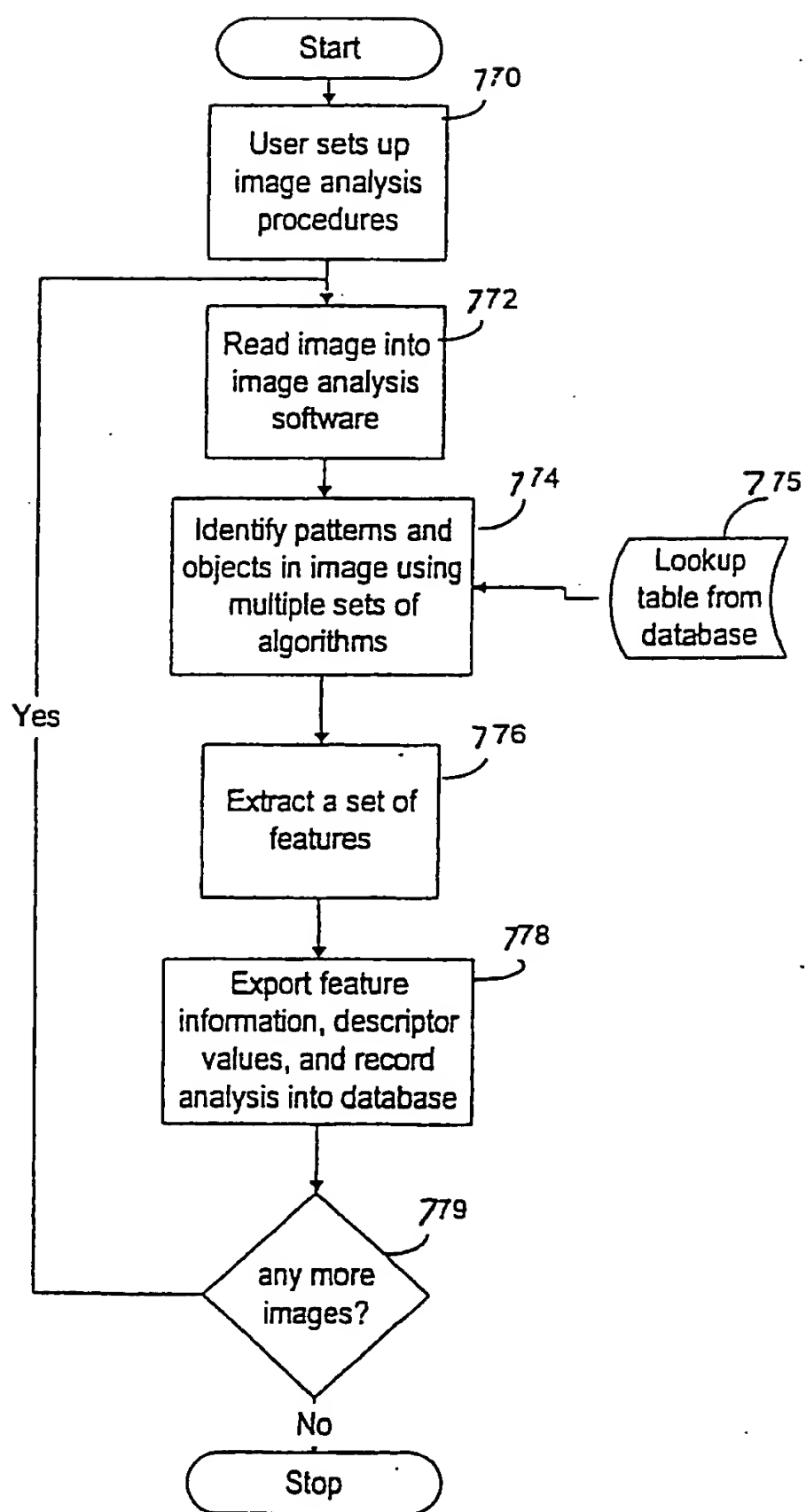


Fig. 71

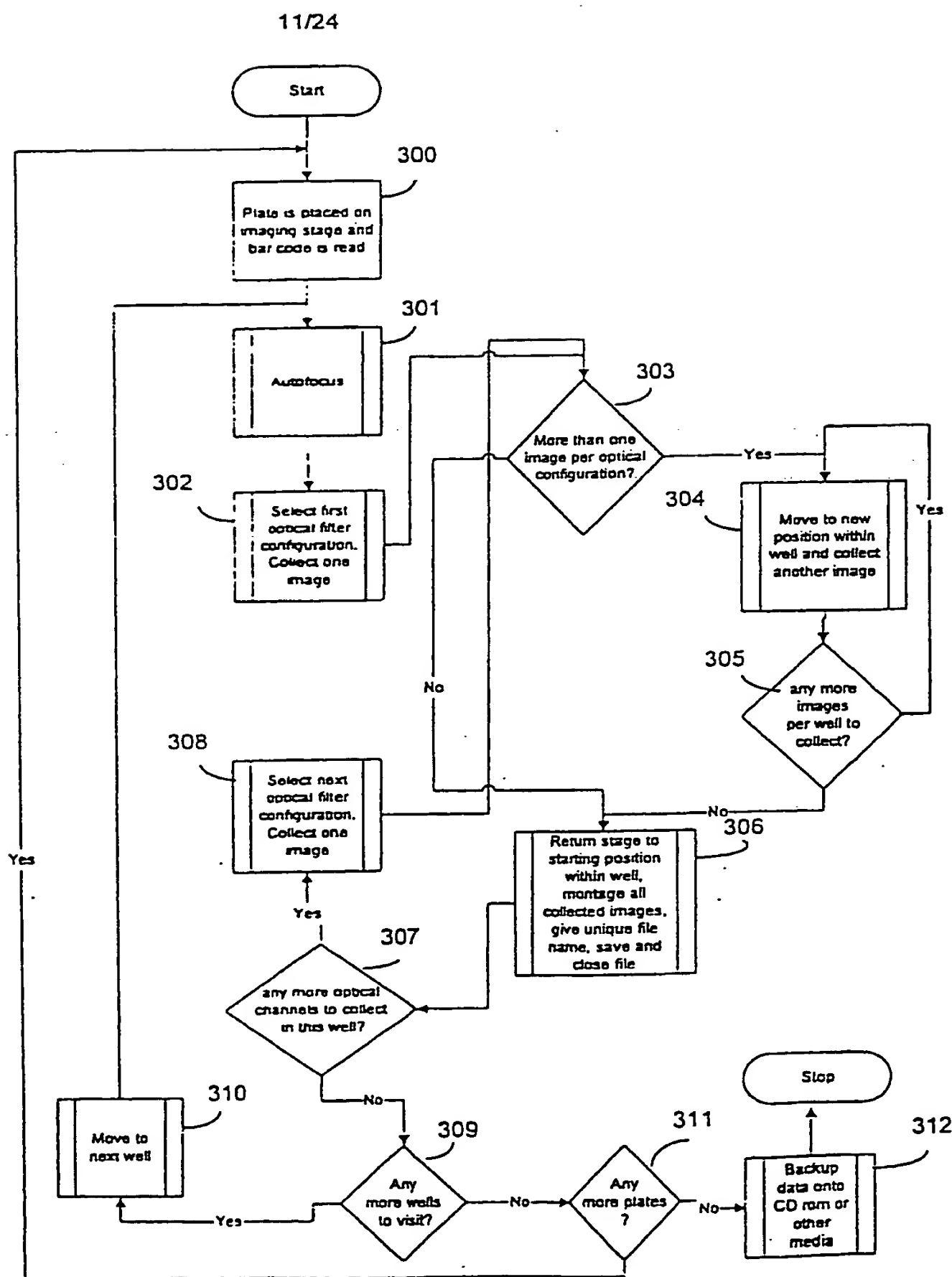


Fig. 7J

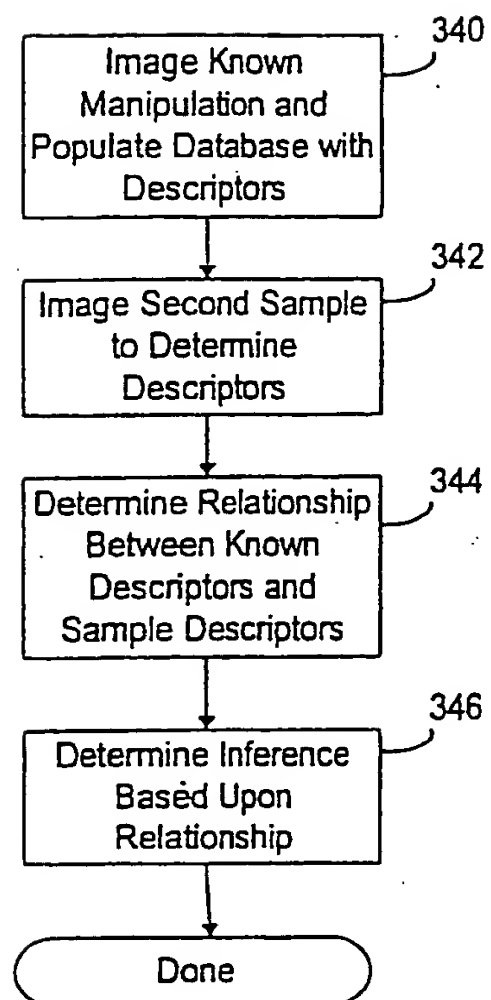


Fig. 7K

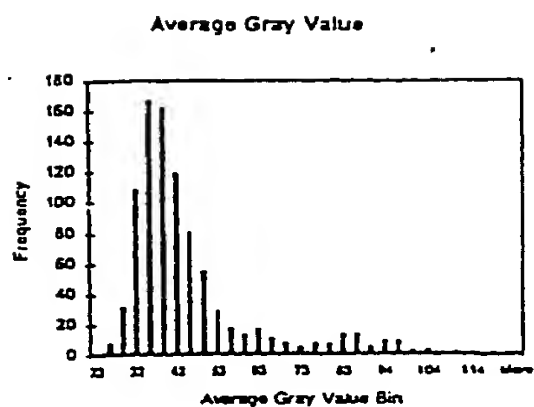


Fig. 8A

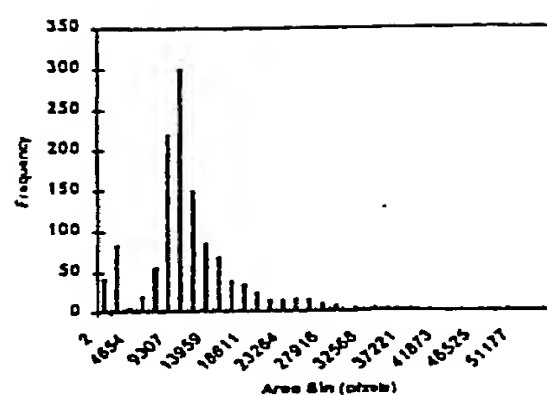


Fig. 8B

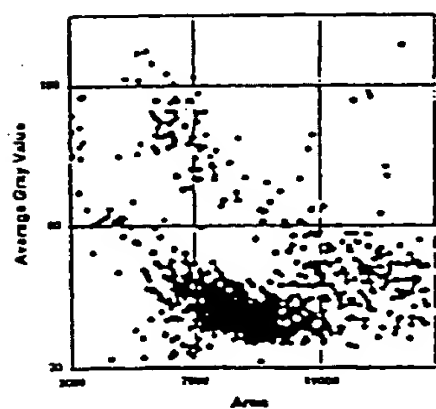


Fig. 8C

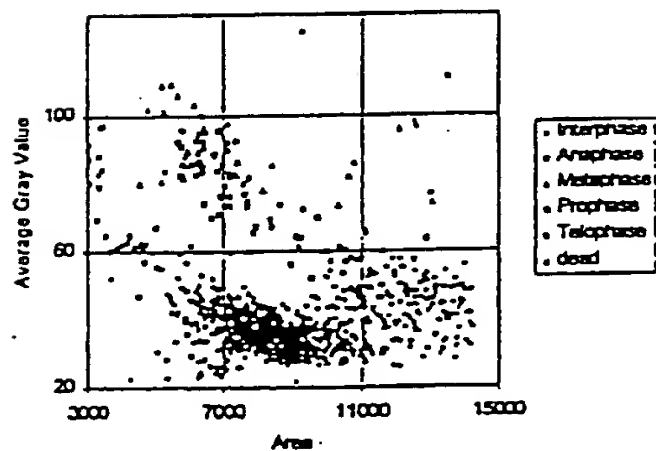


Fig. 8D

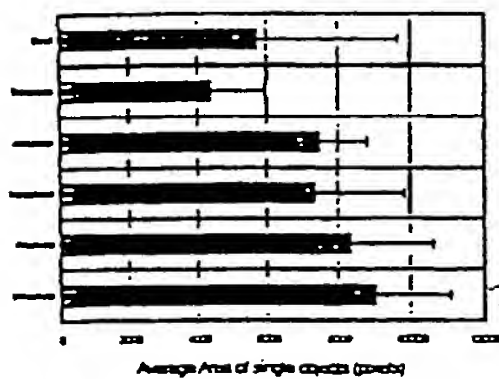


Fig. 8E

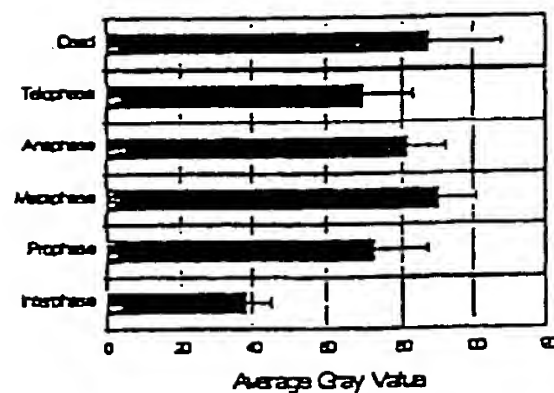


Fig. 8F

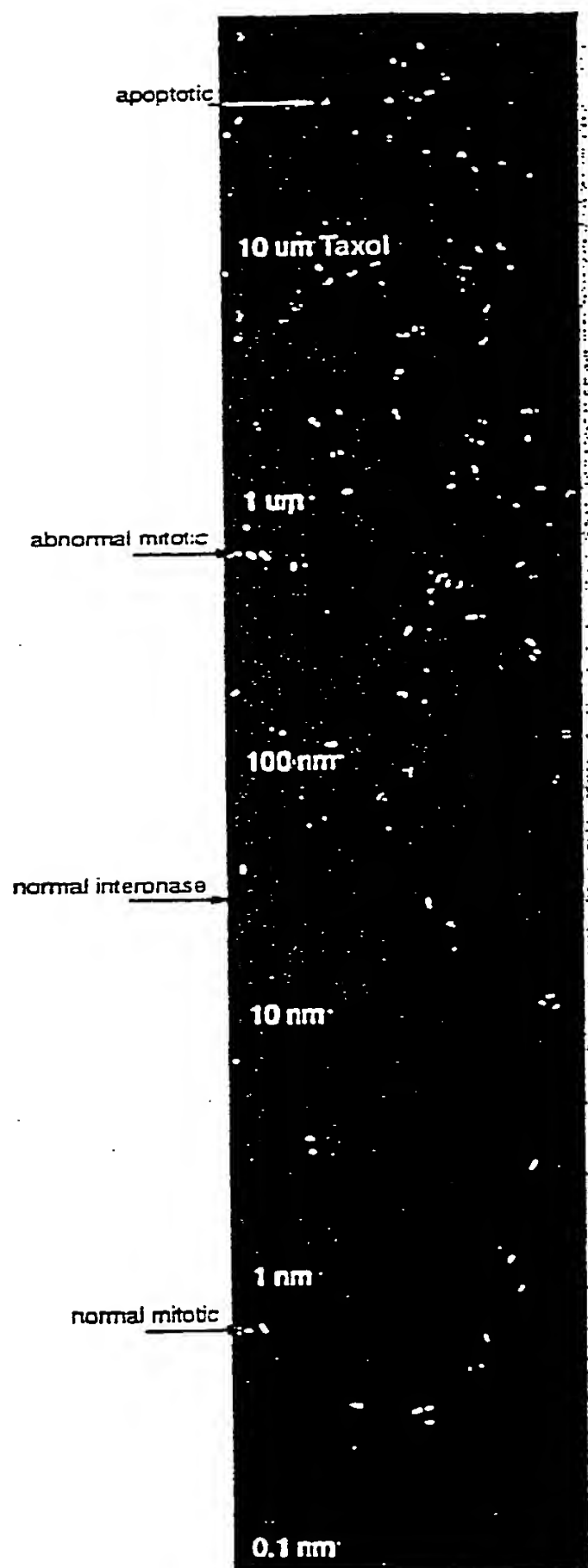


Fig. 9

MDCK cells treated with Taxol for 4.5 hours

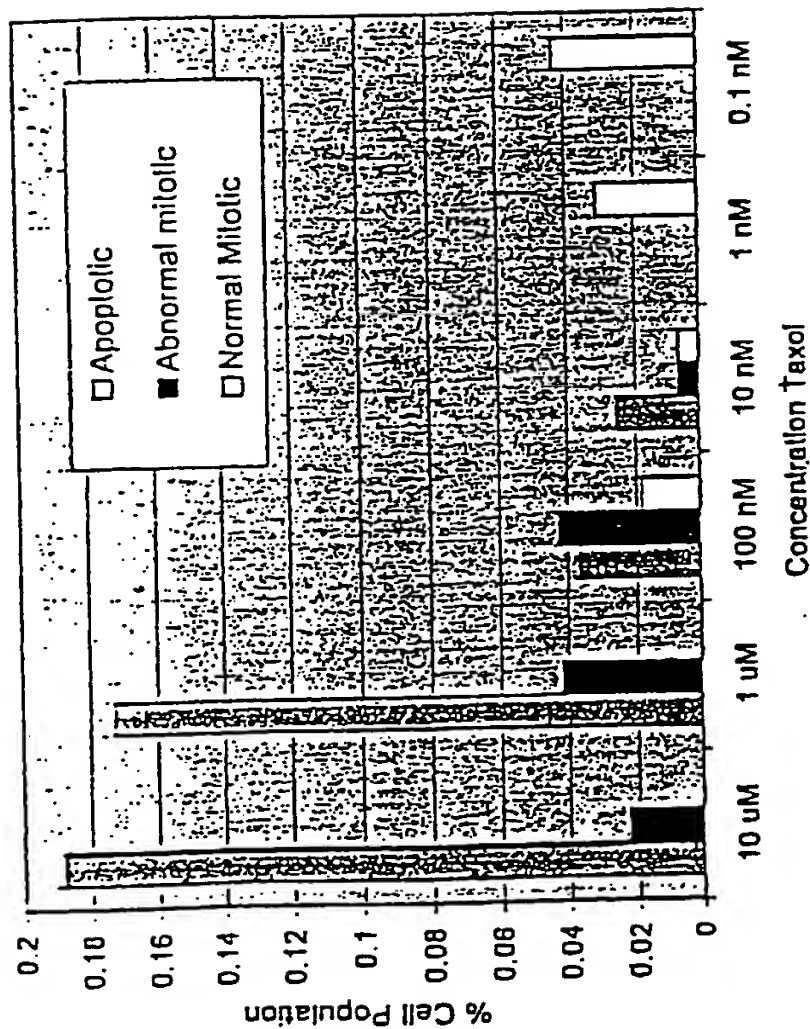


Fig. 10

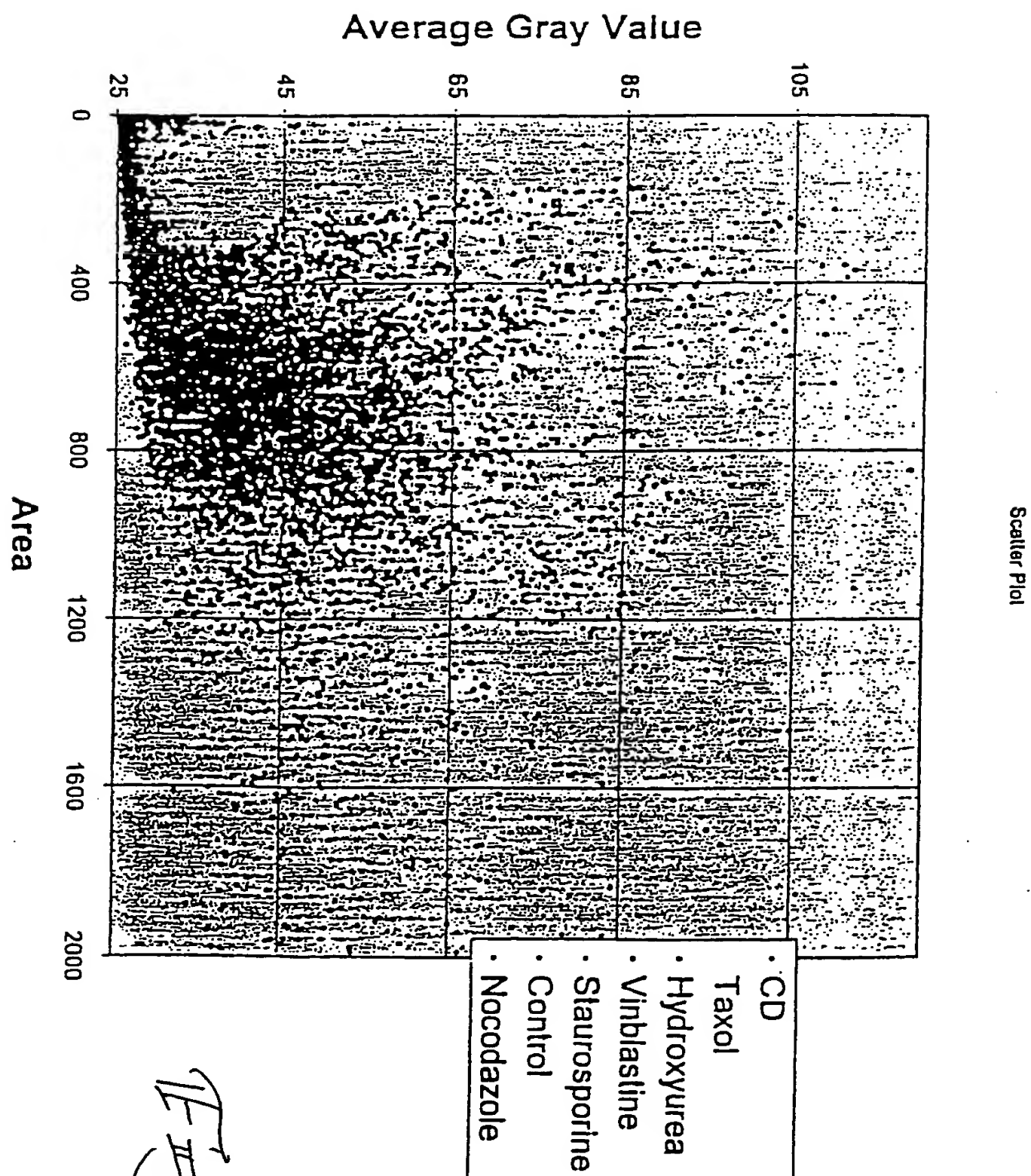
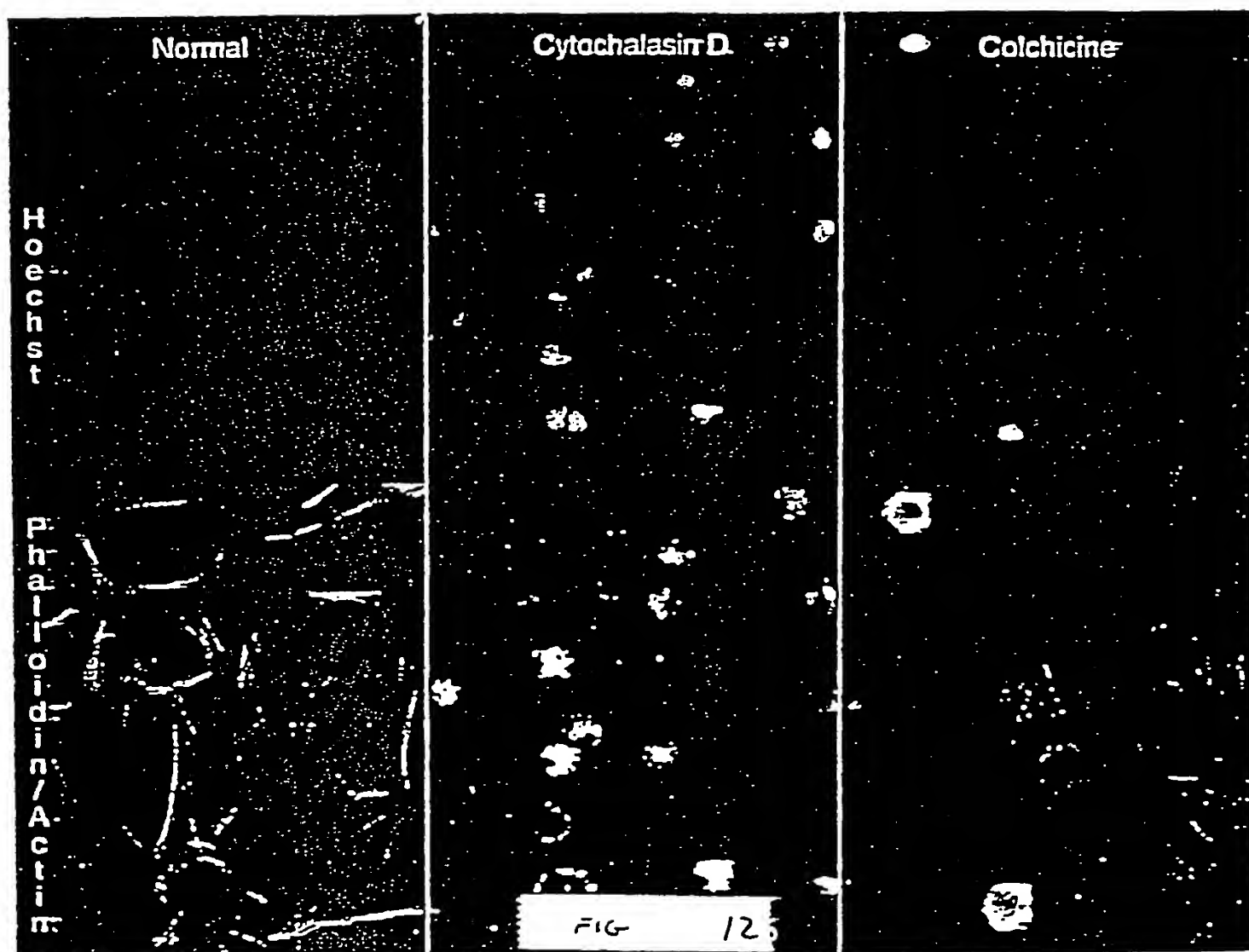


Fig 11



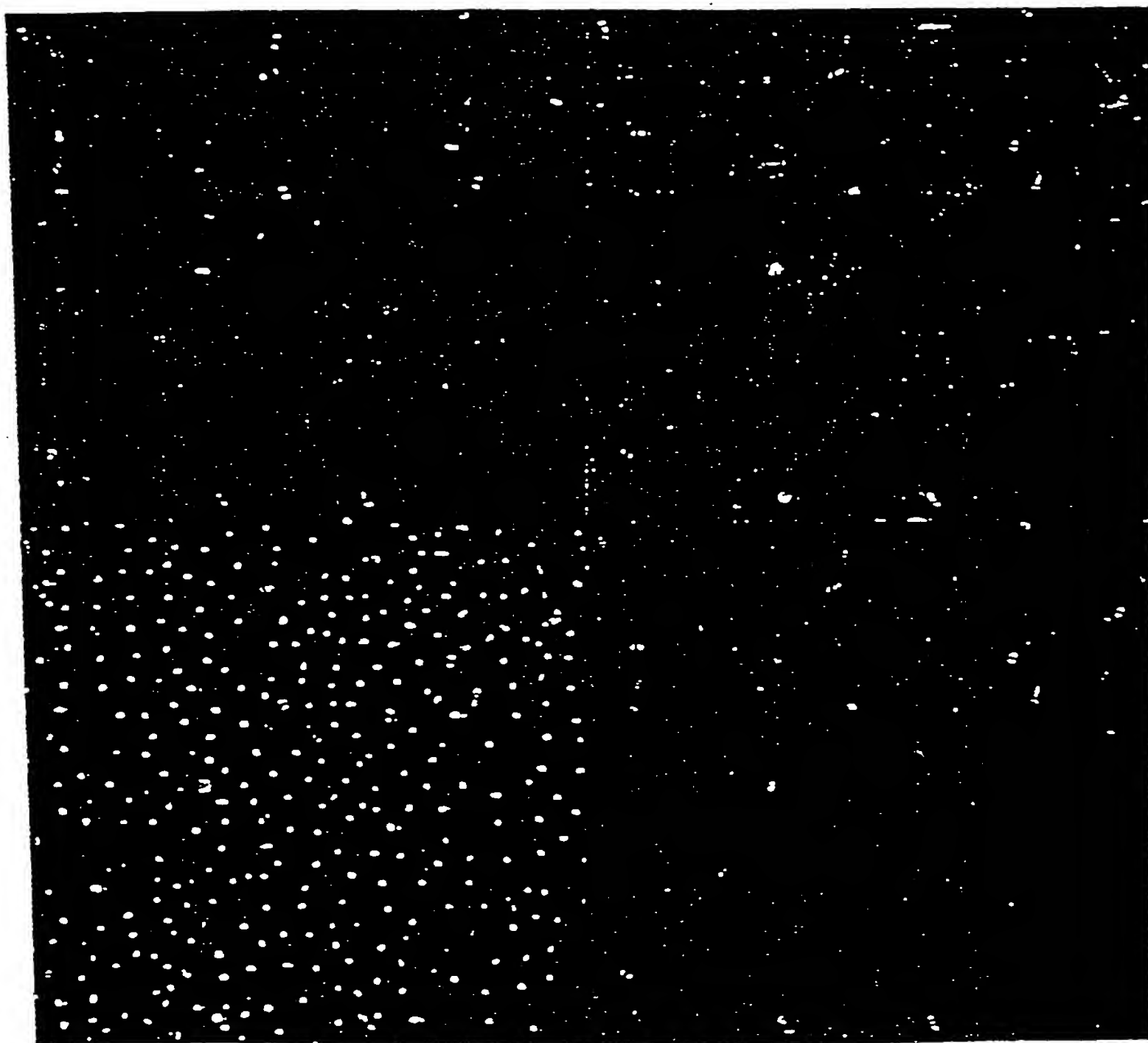


Fig 13

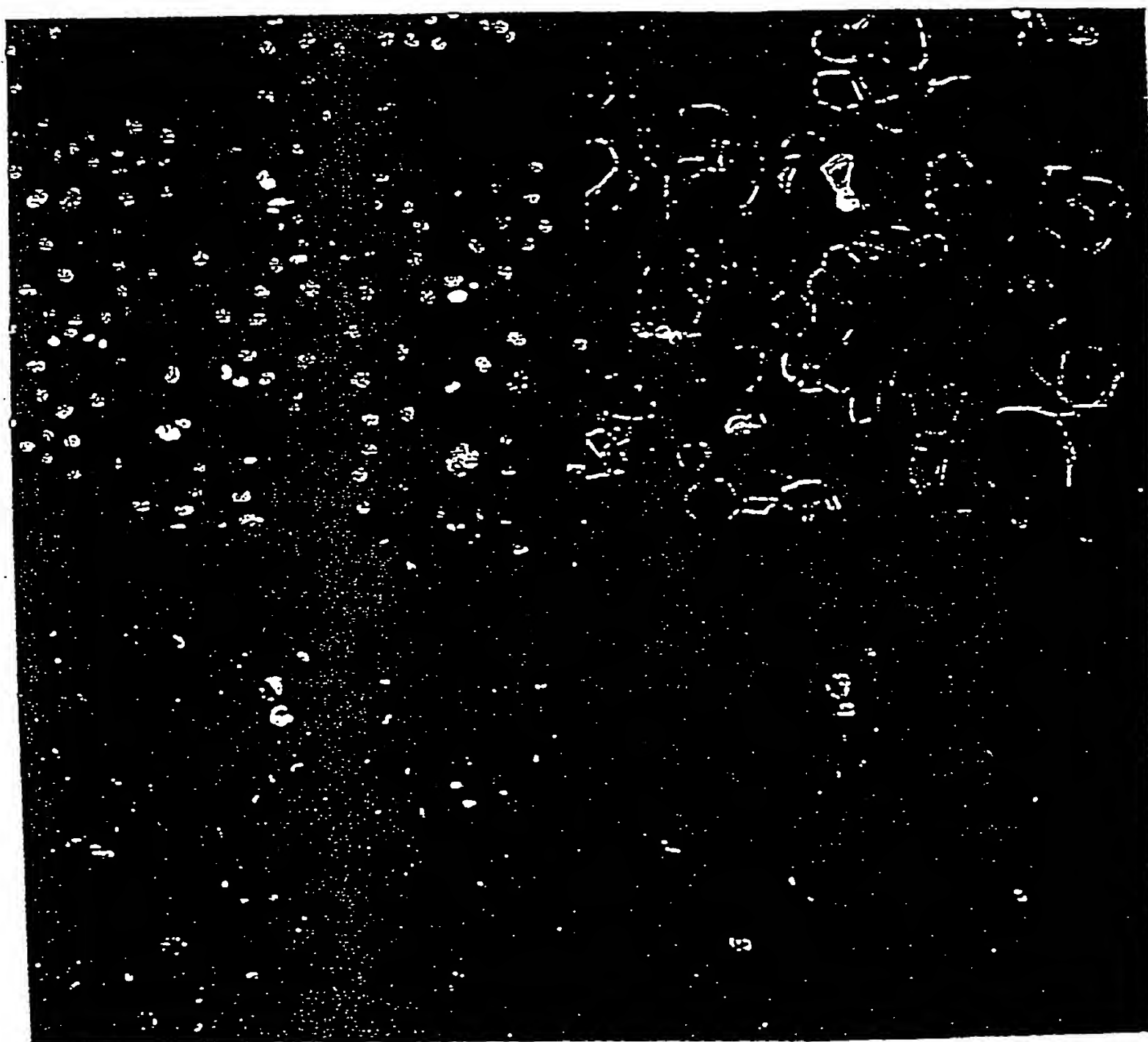


Fig 14

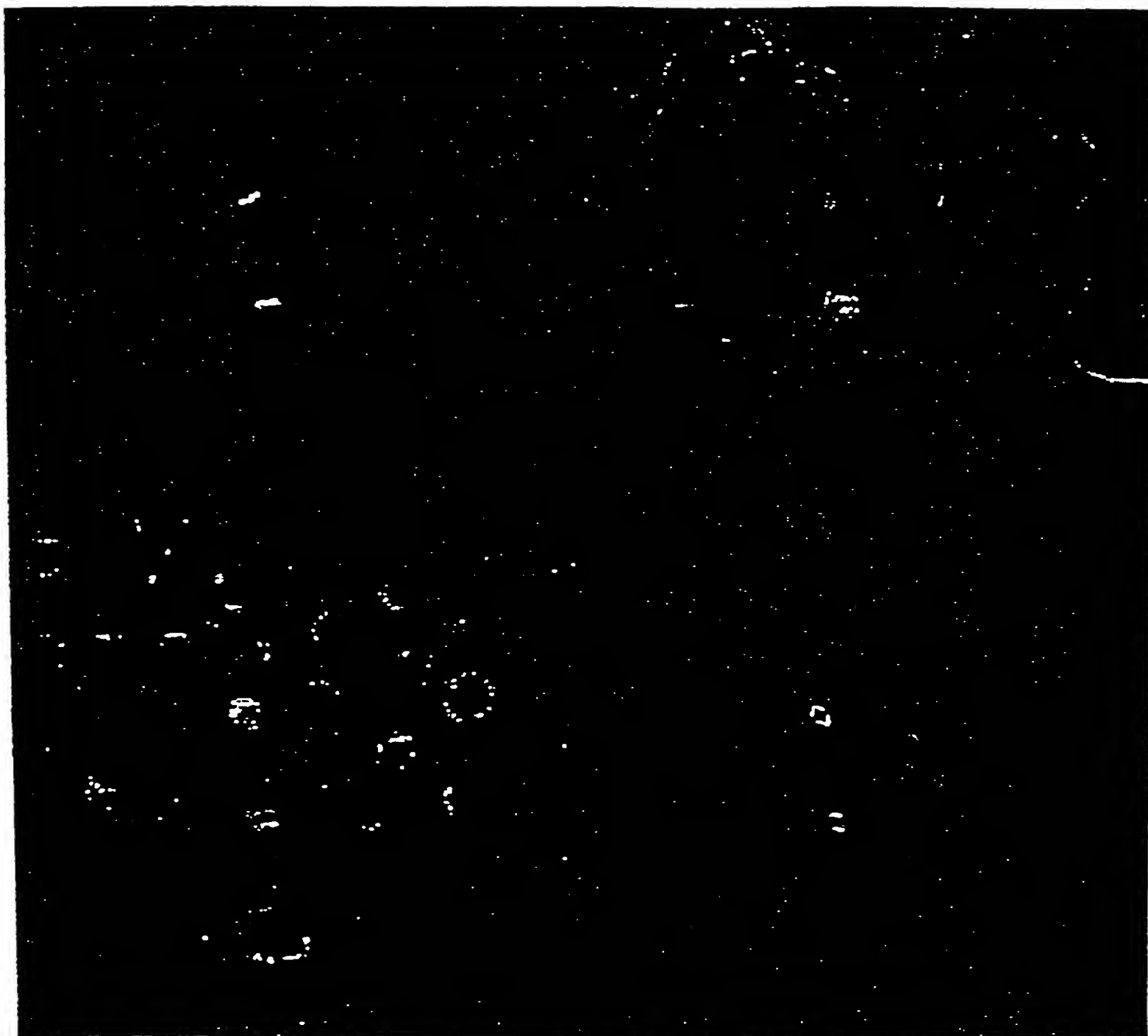


Fig 15

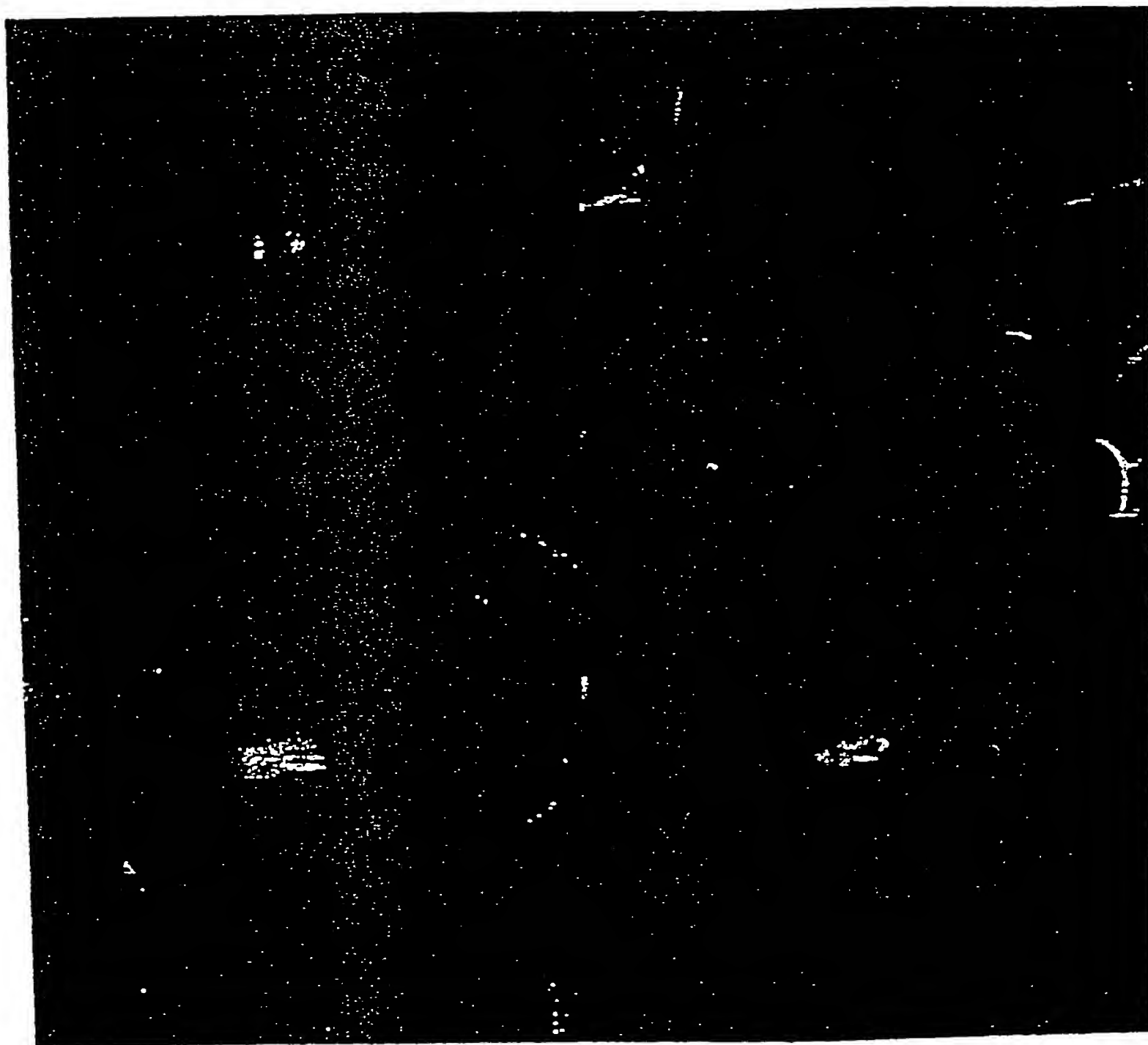


Fig 16



Fig 17

Conversion of morphometric parameters into nucleic acid code
and clustering of the resulting sequences using Neighbor
Joining method.

Compound:	Measurements																			
	Count	Area	Perimeter	Length	Breadth	Fiber length	Fiber breadth	Shape factor	Eli. form factor	Inner radius	Outer radius	Mean radius	Equiv. radius	Equiv. sphere vol.	Equiv. prolate vol.	Equiv. oblate vol.	Equiv. sphere surface area	Average gray value	Total gray value	Optical density
Control	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
Taxol	a	t	t	t	t	t	t	t	a	t	t	t	t	t	t	t	t	t	t	t
CD	c	a	a	a	t	a	t	t	c	a	a	a	a	a	a	a	a	t	a	a
Nocodazole	c	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
Staurosporine	g	g	c	a	a	t	a	a	t	g	a	a	a	t	g	g	g	a	a	t
Vinblastine	c	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	g	t	t
Hydroxyurea	g	t	t	t	t	t	t	g	t	t	t	t	t	t	t	t	t	t	c	t

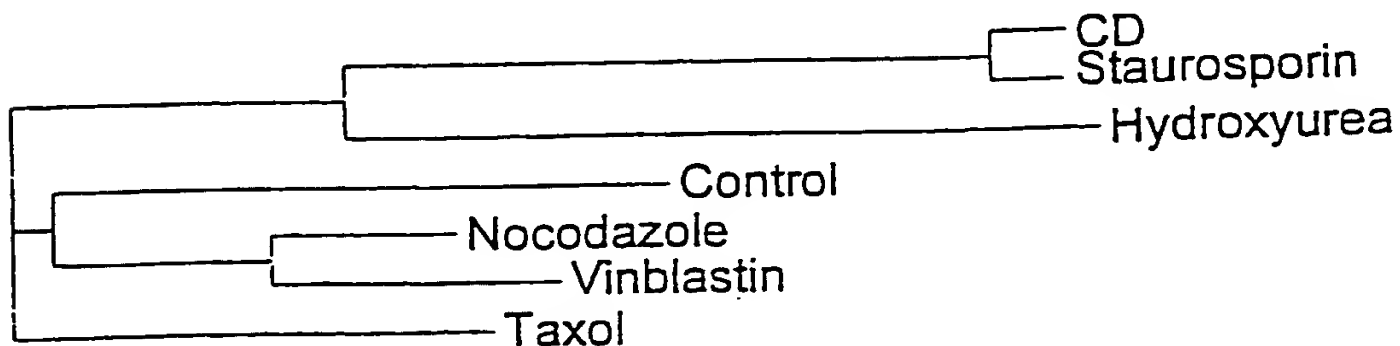


Fig 10

Conversion of morphometric parameters into amino acid codes and clustering of the resulting sequences using Neighbor Joining method.

	Count	Area	Perimeter	Length	Breadth	Fiber length	Fiber breadth	Shape factor	Ell. form factor	Inner radius	Outer radius	Mean radius	Equiv. radius	Equiv. sphere vol.	Equiv. prolate vol.	Equiv. oblate vol.	Equiv. sphere surface area	Average gray value	Total gray value	Optical density	Radial dispersion	Texture Difference Mo	IEFA Harmonic 2, Semi-	IEFA Harmonic 2, Semi-
control	I	P	T	T	Z	S	D	W	M	S	T	T	T	T	C	C	P	P	M	C	T	G	T	T
Taxol	G	F	M	M	P	M	P	H	G	S	M	M	W	C	F	P	T	R	C	M	M	I	M	P
CD	F	G	G	G	M	G	M	K	A	G	G	G	G	G	G	G	G	H	G	G	G	M	G	V
Nocodazol	W	F	M	M	W	M	P	T	R	S	M	M	M	F	M	W	F	M	M	R	M	M	M	F
Staurosporine	N	V	A	G	G	M	G	G	Y	V	G	G	M	V	V	V	V	G	G	H	G	M	G	V
Vinblastine	F	W	W	M	W	W	C	W	D	S	M	W	W	M	M	M	W	M	V	E	M	M	M	P
Hydroxyurea	S	H	H	H	H	H	H	V	H	H	H	H	H	H	H	H	H	H	H	A	H	G	H	D

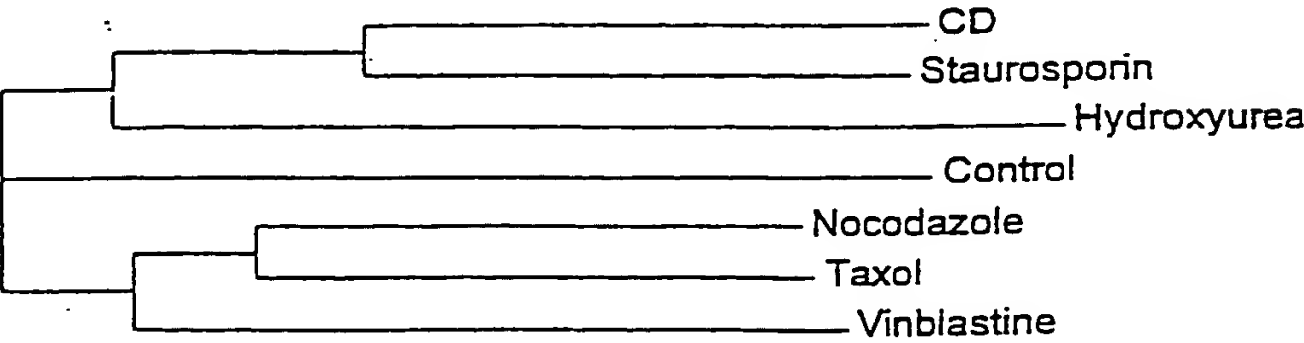


Fig 19

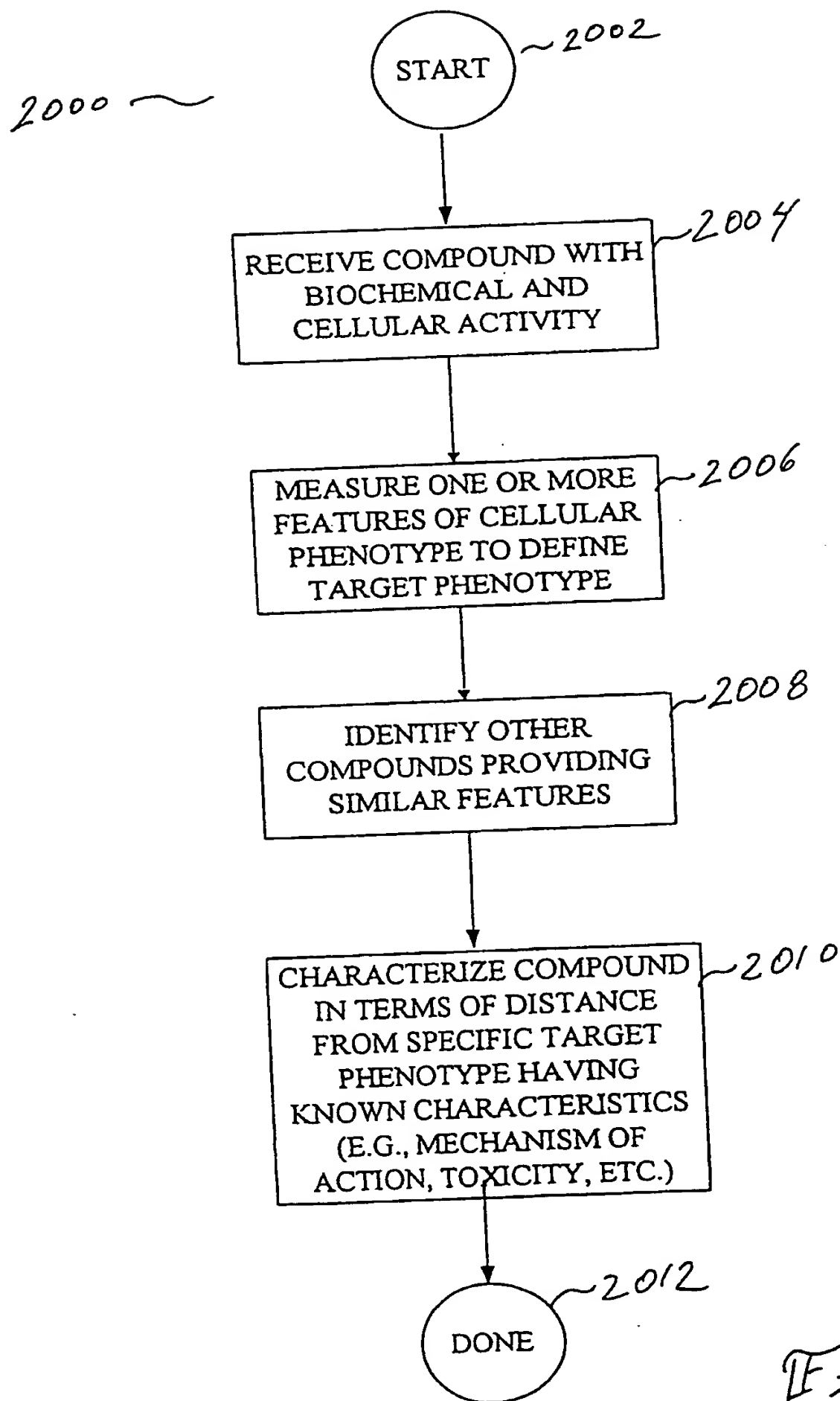


Fig 20

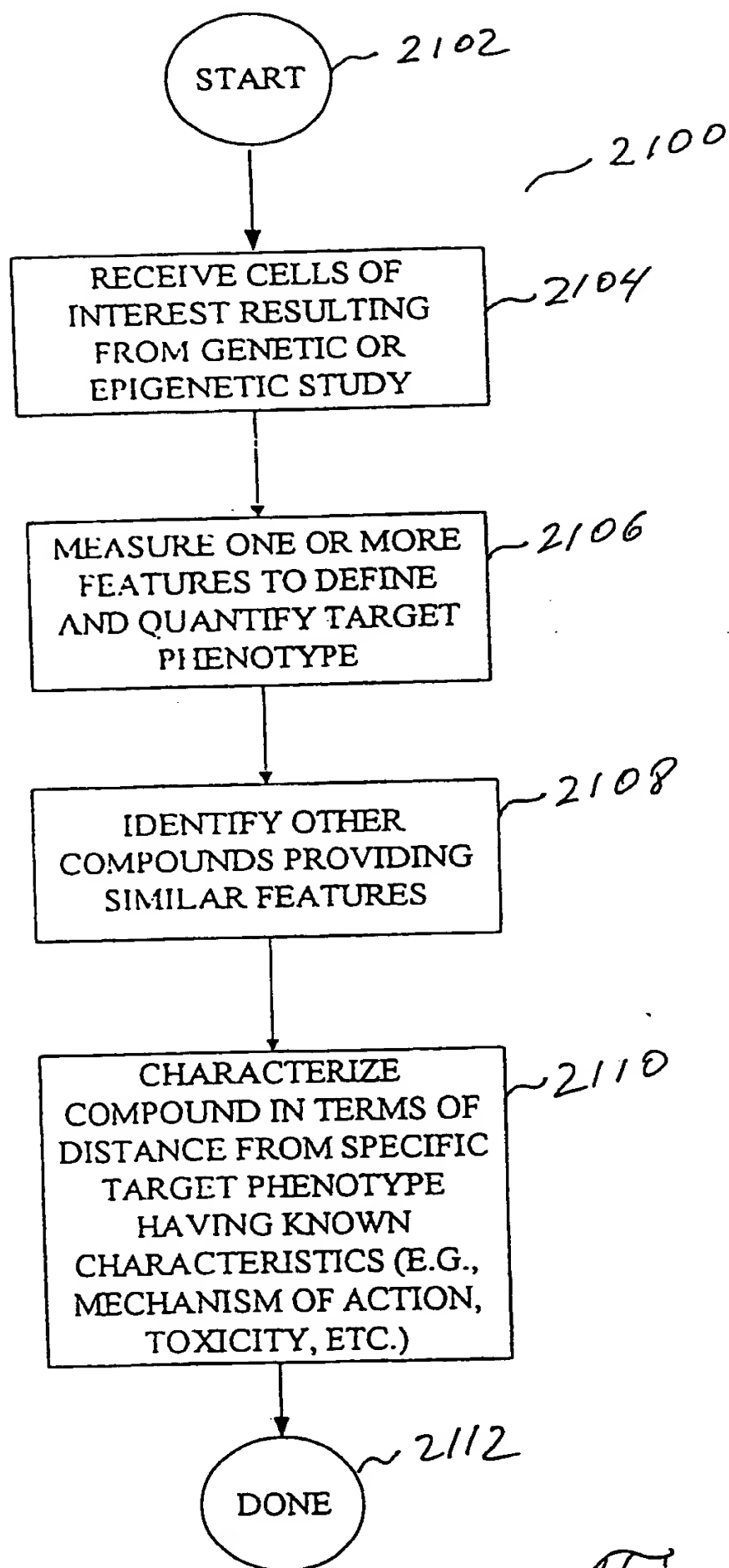


Fig 21

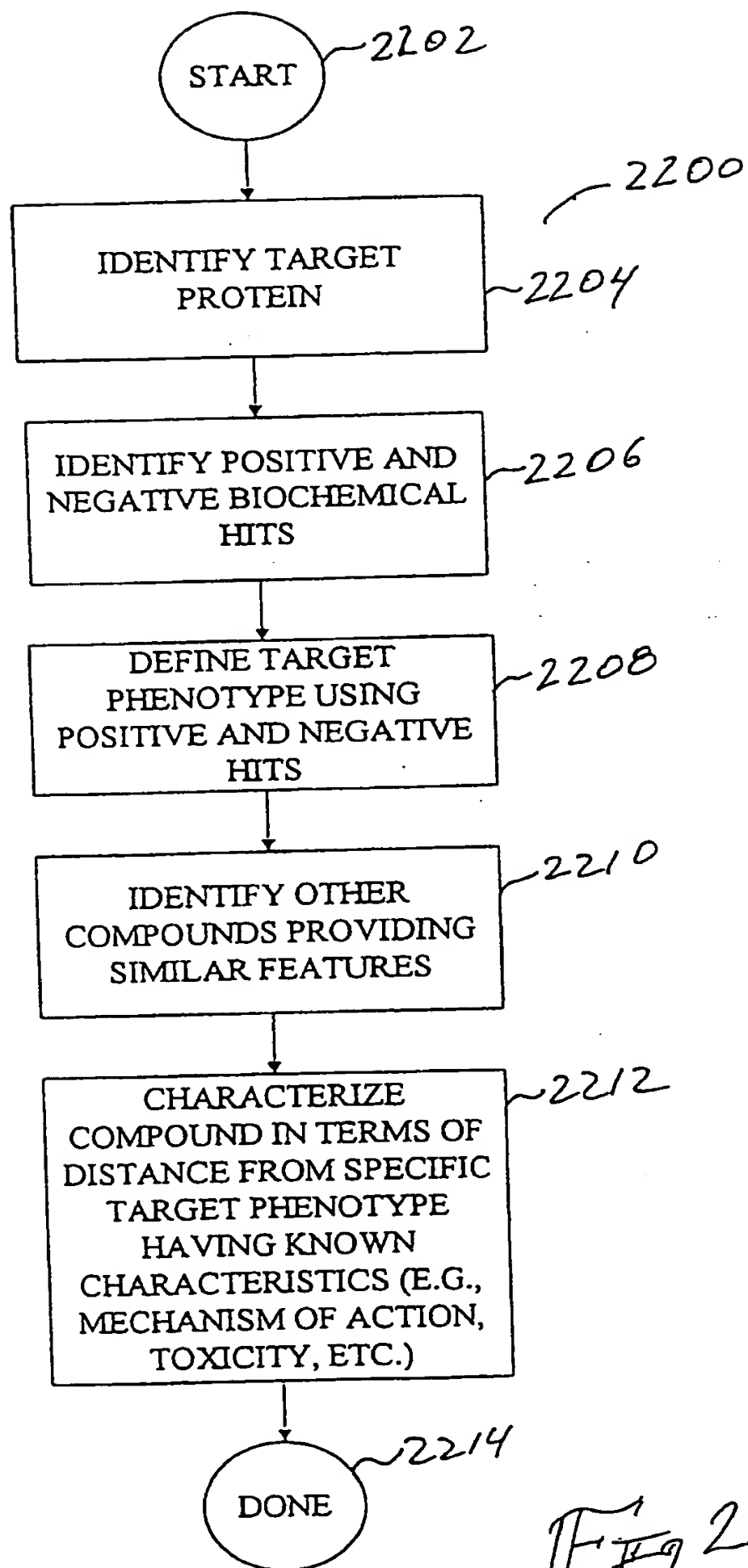


Fig 22